

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Electrochemical Biosensor for Glycated Hemoglobin (HbA1c)

Mohammadali Sheikholeslam, Mark D. Pritzker and Pu Chen
*University of Waterloo
 Canada*

1. Introduction

Diabetes is recognized as a group of heterogeneous disorders with the common elements of hyperglycaemia and glucose intolerance due to insulin deficiency, impaired effectiveness of insulin action or both (Harris & Zimmet, 1997). If left untreated or improperly managed, diabetes can result in a variety of complications, including heart disease, kidney disease, eye disease, impotence and nerve damage. Diagnosis and management of the disease require a tight monitoring of blood glucose levels that serves a number of purposes:

- provides a quick measurement of blood glucose level at a given time.
- determines if a diabetic person has a high or low blood glucose level at a given time.
- demonstrates the link between lifestyle, medication and blood glucose levels.
- helps diabetics and diabetes health-care teams make changes to lifestyle and medication that will improve blood glucose levels.

Electrochemical biosensors for glucose (glucose meters) play a leading role for this purpose. For the purpose of measuring daily glucose levels to control food intake and insulin usage, these glucose meters work although some difficulties exist. For example, blood glucose level measurements are recommended three to four times per day. Due to the large fluctuations in glucose levels that naturally occur over the course of a day, measurements on an empty stomach and within 2 h of eating are required for comparison purposes. These problems are more prominent for the diagnosis of diabetes and determining the link between lifestyle and medication once a patient has been diagnosed with this disease.

Historically, measurement of glucose levels has been the method universally used to diagnose diabetes. Laboratory methods such as fasting plasma glucose (FPG) or 2-h plasma glucose (2HPG) level have been used for this purpose. However, this approach still suffers from the same problems and difficulties associated with glucose biosensors such as the need for fasting, biological variability and the effects of acute perturbations (e.g., stress- or illness-related) on glucose levels. It has recently been concluded that the best marker for long term glycaemic control is whole blood glycated hemoglobin (i.e., hemoglobin A1c denoted as HbA1c) since its levels respond to the long-term progression of diabetes without the short-term fluctuations characteristic of glucose (Berg & Sacks, 2008). Also, the use of this approach solves many of the problems associated with FPG or 2HPG methods based on glucose measurements such as no need for fasting, substantially less biological variability and relative insensitivity of HbA1c levels to acute perturbations. On the other hand with advances in instrumentation and standardization, the accuracy and precision of A1C assays

at least match those of glucose assays. Consequently, the decision was made by the International Expert Committee (with members appointed by the American Diabetes Association, the European Association for the Study of Diabetes, and the International Diabetes Federation) that the A1c assay should be considered as the primary method for the diagnosis of diabetes (Nathan, 2009).

HbA1c is a stable glycated hemoglobin derivative formed by the non-enzymatic reaction of glucose with the N-terminal valine of the β -chain of normal adult Hb (HbA). Since it reflects the average blood glucose level over the preceding 2–3 months and is not affected by the daily fluctuation of the glucose level, the HbA1c level provides a more accurate index for diagnosis and long term control of the disease. Traditionally, clinical laboratory assays for HbA1c have been obtained by ion-exchange chromatography, immunochemical methods, electrophoresis and boronate affinity chromatography. However, these methods are time-consuming, require trained personnel and expensive equipment and have limited availability in many areas of the world. So point-of-care (POC) devices are needed for diabetes diagnosis and management. Point-of-care testing (POCT) is defined as diagnostic testing at or near the site of patient care (Kost, 2002). The driving notion behind POCT is to bring the test conveniently and immediately to the patient. This increases the likelihood that the patient will receive the results in a timely manner. Such devices would allow for immediate availability of A1C measurements and greatly enhance diabetes care. Currently, eight HbA1c POC devices are available commercially with generally accepted performance criteria for HbA1c, but only one of them has met the acceptance criteria of NGSP¹ with two different reagent lots. Also, the reproducibility of production of the different reagent lots of the POC instruments investigated appears inadequate at this moment for optimal clinical use (Lenters-Westra & Slingerland, 2010). As a result, the American Diabetes Association (ADA) recently decided to exclude POC methods from their list of recommended methods for HbA1c diagnosis, stating that they are not yet accurate enough (NGSP, 2010). Also, among these POC instruments, only one is designed for patient use at home, whereas the others are suitable only for clinics and physician offices due to their high price (\$1000–\$3000) and complicated operation. Consequently, considerable work is still needed for the development of accurate, simple and cheap HbA1c biosensors. Although an HbA1c measurement is recommended quarterly and not as frequently as in the case of glucose, its role in prevention, diagnosis and management of diabetes is critical.

2. Electrochemical biosensors

A biochemical sensor is a small device consisting of a transducer covered by a biological recognition layer which interacts with the target analyte. The chemical changes resulting from this interaction are converted by the transducer into electrical signals. Electrochemical biosensors combine the analytical power of electrochemical techniques with the specificity of biological recognition processes to produce an electrical signal that is related to the concentration of an analyte (Wang, *Analytical Electrochemistry*, 2006). In electrochemical biosensors, the transducer is an electrode. Based on the nature of the biological recognition process, two general categories of electrochemical biosensors can be defined: biocatalytic devices (utilizing enzymes, cells or tissues as immobilized biocomponents) and affinity

¹ National Glycohemoglobin Standardization Program

sensors (based on antibodies, membrane receptors or nucleic acids) (Wang, *Analytical Electrochemistry*, 2006). Electrochemical biosensors can be further divided into the sub-categories of potentiometric, amperometric and impedimetric biosensors depending on their mode of operation (Pohanka & Skládal, 2008). Electrochemical biosensors are widely used in the medical field. One of the most important applications of such devices is for the diagnosis and management of diabetes, a topic which has received a great deal of interest due to its urgent need and as a model system for sensor development.

2.1 Glucose biosensors

Glucose biosensors are one of the key elements in treating and management of diabetes. Many diabetics use these devices to measure their blood glucose level every day. In fact, glucose biosensors occupy 85% of the entire biosensor market. Such huge market size has made diabetes a model disease for developing new biosensing concepts (Wang, *Electrochemical Glucose Biosensors*, 2008). It has been about 36 years since the first commercial glucose biosensor was introduced into the commercial market (Pohanka & Skládal, 2008). From that date, different approaches have been explored and many devices have been designed for individual diabetes control. In spite of the huge development in glucose biosensors, diabetes control still has problems and so efforts are still being made to further improve their use. Issues such as *in vivo* glucose measurement and insulin delivery and long-term glucose level measurement are some areas of interest. As mentioned previously, the problems associated with the measurement of long-term blood glucose levels are leading to the development of HbA1c biosensors. HbA1c biosensors integrated with personal glucose biosensors can greatly improve management and treatment of diabetes.

3. HbA1c biosensors

3.1 Biosensors based on Fructosyl Valine (FV)

As mentioned previously, the problems associated with the measurement of long-term blood glucose levels are leading to the development of HbA1c biosensors. HbA1c biosensors integrated with personal glucose biosensors can greatly improve management and treatment of diabetes. As mentioned previously, HbA1c is formed through the non-enzymatic glycation of the terminal valine of beta sheets in hemoglobin. This HbA1c can be digested to small glycated peptide fructosyl valine (FV) that can be further oxidized by the enzyme fructosylamine oxidase (FAO). Enzymatic assay of HbA1c is based on the oxidation of FV (as a model compound).

In one of the first studies on FV enzyme sensors, Sode et. al. used an isolated fructosyl amine oxidase from marine yeast (Tsugawa, Ishimura, Ogawa, & Sode, 2000). They fabricated 2 types of sensors: a mediator-type enzyme sensor (using carbon paste electrode) and a hydrogen peroxidase-based enzyme electrode. Although lower potentials (150 mV vs. Ag/AgCl) were applied for the mediator-type probe than for the other one (600 mV vs. Ag/AgCl), the sensitivity of the hydrogen peroxidase sensor was found to be higher (0.42 $\mu\text{A mM}^{-1} \text{cm}^{-2}$). Consequently, further optimization of the operating conditions was needed as well as the sensor design. In a subsequent study, this group developed an FAO-peroxidase-ferrocene sensor and a Prussian blue-based FAO sensor (Tsugawa, Ogawa, Ishimura, & Sode, 2001). The sensitivities of these probes were found to be similar to that of the earlier hydrogen peroxidase sensor but the applied potentials were lowered dramatically

(−250 mV and −50 mV for FAO-peroxidase-ferrocene sensor and Prussian blue-based FAO sensor, respectively). However the linear range of the current-concentration calibration curves for both sensors was narrower and the response times were longer than in the case of the hydrogen peroxidase sensor.

Molecular imprinting is a technique to create template-shaped cavities in polymer matrices with memory for the template molecules to be used in molecular recognition (Alexander, et al., 2006). Sode et. al. employed a synthetic polymer (polyvinylimidazole denoted as PVI) as a catalyst for fabrication of an amperometric FV sensor (Sode, Takahashi, Ohta, Tsugawa, & Yamazaki, 2001). They combined this catalytic center with molecular imprinting for oxidative cleavage of FV. In their method, a mixture of carbon paste and PVI was applied on the electrode. The constructed electrode was then immersed in the phosphate buffer electrolyte containing m-PMS as mediator (Fig. 1). The current for the anodic oxidation of the reduced mediator (resulting from oxidation of FV) was monitored after applying an electrode potential of +100mV vs. Ag/AgCl. This system showed a linear relation between the current and the fructosylvaline concentration over the range from 50μM to 10mM in the presence of 5mg/ml PVI. Fig. 2 shows an excellent linear response of the current over a FV concentration range from 20μM to 0.7mM. Although the electrode sensitivity was not reported, they reported a detection limit for the sensor of about 20μM, which is acceptable for diabetes diagnosis, and measurement reproducibility within 10%. Also, the current response of a bare carbon electrode was found to be about 15% of that obtained in the presence of the PVI catalyst.

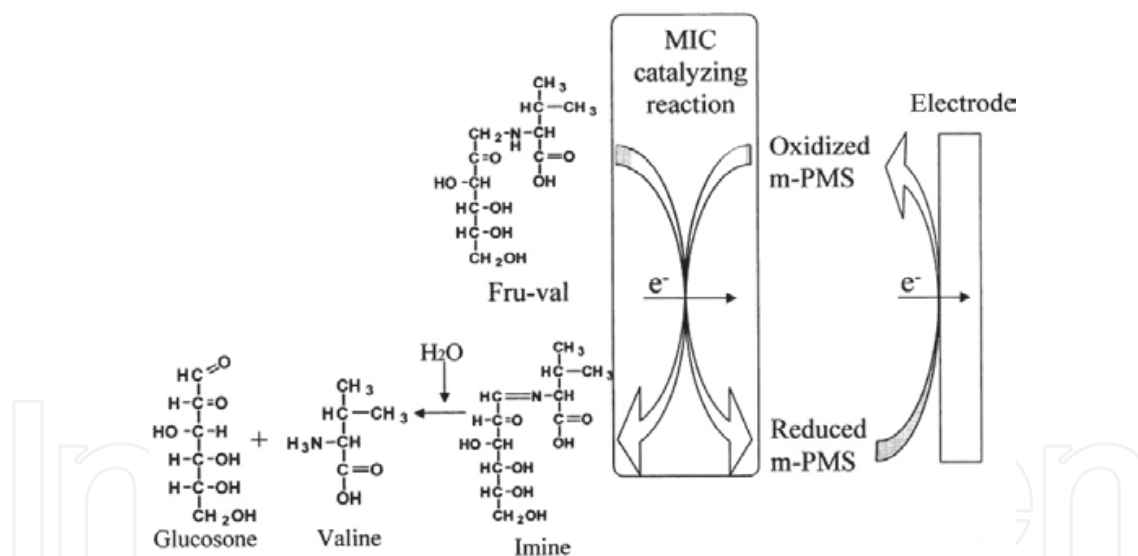


Fig. 1. Oxidative fructosylamine cleavage reaction and detection on MIC-employing electrode (Sode, Ohta, Yanai, & Yamazaki, 2003).

Since FV is an expensive reagent, it is the limiting factor for its utilization as the template for sensor fabrication. Proteolytic digestion of HbA1c for production of FV also leads to the formation of another fructosylamine compound (fructosyl lysine denoted as Fru-ε-lys) which is the proteolytic product of digestion of glycated albumin in the blood and can interfere with the detection of FV. So Sode and coworkers developed a sensor for better selectivity for FV over fructosyl lysine and used methyl valine (m-val) which is a cheaper analogue of expensive FV as the template (Sode, Ohta, Yanai, & Yamazaki, 2003). Also, they used the positively charged functional monomer allylamine to improve the selectivity of the

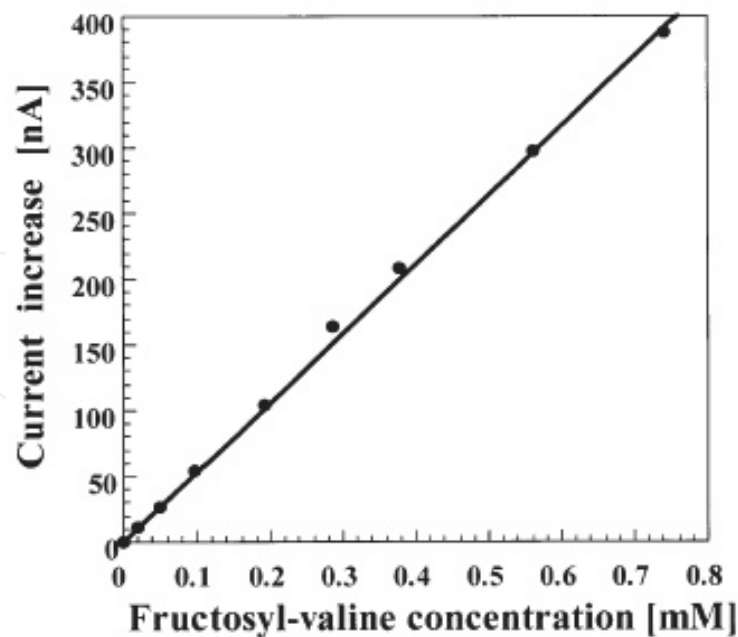


Fig. 2. Calibration curve for amperometric fructosylamine sensor employing PVI as the catalyst. The measurements were carried out in 10mM potassium phosphate buffer (pH 7.0) containing 1mM m-PMS at 50°C (Sode, Takahashi, Ohta, Tsugawa, & Yamazaki, 2001).

sensor toward FV. Both the sensitivity and selectivity (FV/Fru-ε-lys) decreased from 135nA/mM to 95nA/mM and 1.8 to 1.6, respectively, after replacing the FV template with m-val. However, with the introduction of allylamine as the functional monomer, the selectivity increased to 1.9, while a sensitivity of 95nA/mM could be maintained. Thus, with these two modifications, selectivity increased slightly, while the sensitivity decreased in exchange for a more inexpensive template (m-val). Table 1 shows a comparison of the sensitivities and selectivities achieved by the use of different polymers in their study.

Polymer	Template	Allylamine	Sensitivity (nA/mM)		Selectivity (Fru-val/Fru-ε-lys)
			Fru-val	Fru-ε-lys	
P1	—	—	79 ($R^2 > 0.984$)	70 ($R^2 > 0.988$)	1.1
P2	Fru-val	—	135 ($R^2 > 0.992$)	75 ($R^2 > 0.982$)	1.8
P3	m-val	—	95 ($R^2 > 0.984$)	60 ($R^2 > 0.968$)	1.6
P4	m-ε-lys	—	84 ($R^2 > 0.980$)	101 ($R^2 > 0.976$)	0.8
P5	—	+	91 ($R^2 > 0.996$)	54 ($R^2 > 0.998$)	1.7
P6	m-val	+	95 ($R^2 > 0.992$)	50 ($R^2 > 0.980$)	1.9

Table 1. Sensitivity and selectivity of polymers for fructosylamine compounds (Sode, Ohta, Yanai, & Yamazaki, 2003).

Fang and his coworkers developed a single-use, disposable fructosyl valine amperometric biosensor. Since HbA1c measurement is vital for long-term management of diabetes in patients, a cheap single-use disposable HbA1c sensor could be very useful in this regard. They used screen-printed electrodes for sensor fabrication and incorporated iridium into the electrodes as a catalyst (Fig. 3). Both the working and counter electrodes were iridium-modified carbon, while the reference electrode was Ag/AgCl. Fructosyl amine oxidase

(FAO) was immobilized on the working electrode for detection of H_2O_2 produced enzymatically from FV in a 3 μL sample. Amperometric measurements were done in a medium containing PBS, FV and potassium chloride as the supporting electrolyte at pH 7.0 and room temperature for 120 seconds after applying an electrode potential of +0.25 V at HbA1c concentrations from 0 to 2 mM that correspond to the range relevant to physiological conditions. The results are shown in Fig. 4 and Fig. 5. Fang et al claimed a sensitivity of 21.5 $\mu\text{A mM}^{-1} \text{cm}^{-2}$ for their sensor which is several orders of magnitude larger than the value reported in the physiological range. At the same time, their applied potential of +0.25 V was lower than that used in most previous studies on this type of sensor. However their FV samples were synthesized using L-valine and glucose and so should not have experienced the potential interferences due to the presence of the proteolytic products of HbA1c other than FV.

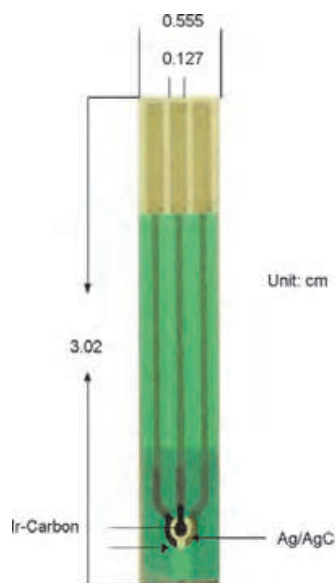


Fig. 3. The configuration of the thick-film sensor (Fang, Li, Zhou, & Liu, 2009).

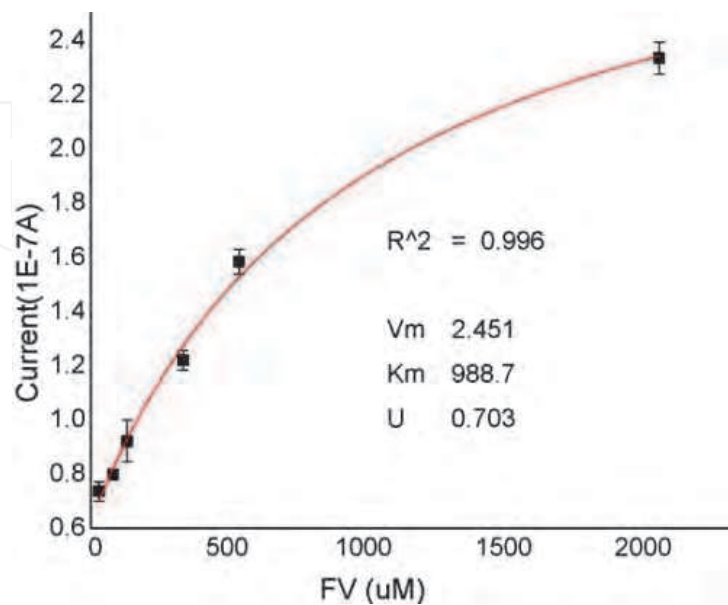


Fig. 4. Calibration curve for the FV biosensor (Fang, Li, Zhou, & Liu, 2009).

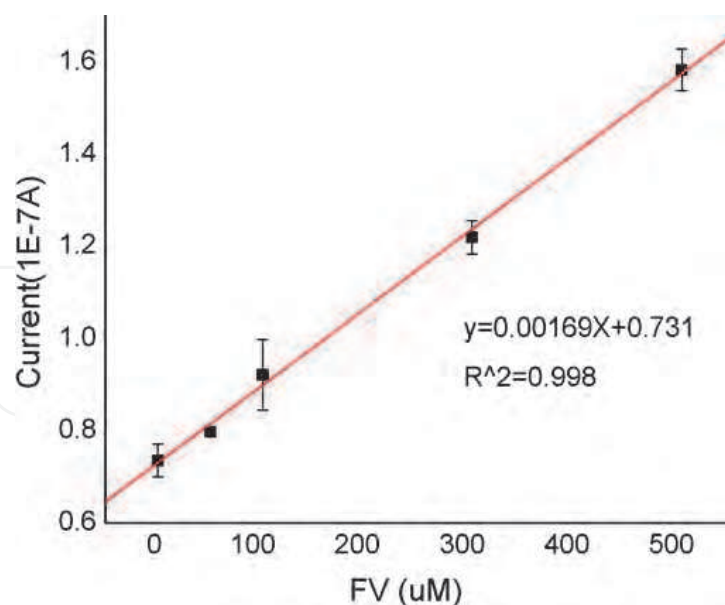


Fig. 5. Calibration curve for the FV biosensor at concentrations below 1mM FV (Fang, Li, Zhou, & Liu, 2009).

In another study, Chuang et. al. used the same technique of molecular imprinting to fabricate a potentiometric FV biosensor (Chuang, Rick, & Chou, 2009). They made molecular imprints of FV in a poly-aminophenylboronic acid (p-APBA) polymer on conductive indium-doped tin oxide (ITO) electrodes. Electrochemical characterization of the fabricated biosensor was carried out by comparing the open circuit potential (E_{oc}) of the ITO carrying the molecular imprinted polymer (MIP) with that measured on a non-imprinted control in 10mL phosphate buffer (pH 7.0) with a standard Ag/AgCl reference electrode to assess the affinity of the FV imprints for FV, D-fructose, D-glucose and L-valine. The ΔE_{oc} values obtained when the imprinted electrode was introduced into solutions containing 10 mM FV, 10 mM D-fructose, 10 mM D-glucose and 10 mM L-valine were found to be $\sim 5.0 \times 10^{-3}$ V, $\sim 2.9 \times 10^{-3}$ V, $\sim 4.0 \times 10^{-4}$ V and less than 1.0×10^{-5} V, respectively. The higher ΔE_{oc} values measured in the presence of D-fructose than D-glucose indicates that the electrode recognises the limited structural similarity between D-fructose and D-glucose. Also, it is apparent that the affinity of the imprinted electrode for FV is higher than for the others. The suggestion was made that this may be due to both shape complementarity (as evident in the case of D-fructose and D-glucose) and charge effects. The p-APBA polymer has a net positive charge in pH 7.0 buffer while FV is negatively charged. Selectivity through shape recognition was attributed mainly to the imprinting of the carbohydrate component of FV, as suggested from a comparison of the ΔE_{oc} values for fructose, glucose and valine.

Electrochemical oxidation of FV on a bare glassy carbon paste electrode (GCPE) in the absence of an enzyme was reported by Chien et. al (Chien & Chou, 2010). The electrode was prepared by applying a glassy carbon microparticle paste on the ITO substrate using a baking process. This GCPE was characterized and reported to have higher sensitivity on FV and lower background current compared with conventional glassy carbon electrodes. After studying the polarization behaviour of FV to determine an appropriate applied potential that would yield higher sensitivity and signal-to-noise ratio (+0.1 V), the current response of the GCPE to successive additions of FV (0 to 1mM) was collected by chronoamperometric measurement in a phosphate buffer (pH 7.4) (Fig. 6). The average response time and

stabilization time between each addition was found to be 40 and 100 seconds, respectively. According to the data (correlation between FV concentration and the response current in phosphate buffer) shown in the inset of Fig.6, the response current increased from 0.27 μA to 5.52 μA as the FV concentration increased from 0 to 1.0 mM. These data also show a good linearity with an R^2 value of 0.999. The sensitivity of the biosensor was found to be 5.26 $\mu\text{A mM}^{-1}$ and the minimum detection limit less than 0.1 mM. Chien et al. also showed that an increase in pH leads to a rise in the oxidation current. Moreover, the biosensor exhibited a high selectivity for FV as D-glucose, D-fructose and L-valine had no interference on the current response. However, it should be acknowledged that their FV samples were synthesized using L-valine and glucose so the use of a high applied potential may cause interference and necessitate the inclusion of a mediator in future applications.

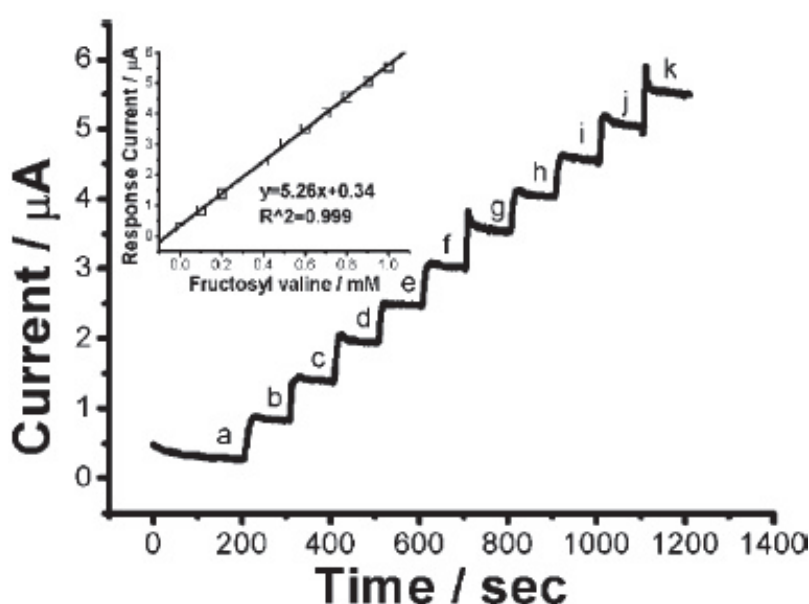


Fig. 6. Chronoamperometric response at GCPE (glassy carbon microparticles/mineral oil 50/50 (w/w) %). FV concentrations, increasing in mM L^{-1} increments, are shown as: (a) 0, (b) 0.1, (c) 0.2, (d) 0.3, (e) 0.4, (f) 0.5, (g) 0.6, (h) 0.7, (i) 0.8, (j) 0.9, (k) 1. The current readings were observed to stabilize for approximately 100s. The supporting electrolyte was phosphate buffer (pH 7.4), the operating potential was +1.0 V (vs. Ag/AgCl) with the measurements being made at ambient temperature. Inset: Calibration curve obtained for different concentrations of FV (Chien & Chou, 2010).

3.2 Biosensors based on HbA1c

Other types of HbA1c biosensors detect HbA1c directly. Different methods and techniques have been applied for these types of HbA1c biosensor. One of their potential advantages is that there is no need for two time-consuming preliminary steps to release fructosyl valine from HbA1c by a protease (one of the main drawbacks with FV POC instruments).

Stöllner et al. developed an immunoenzymometric assay (IEMA) in which a glycated pentapeptide (with an amino acid sequence corresponding to the first 5 amino acids of the N-terminal hemoglobin sequence of the beta-chain) was used as an HbA1c analogue on the surface of either a microtiter plate or an amino-modified cellulose membrane (Stöllner, Warsinke, Stöcklein, Dölling, & Scheller, 2001). In this sensor, this glycated peptide

competes with HbA1c in the sample for antigen binding sites of anti-HbA1c. After a washing step, a glucose oxidase-conjugated antibody is applied to indicate the previously bound antibodies to the glycated peptide. Then the bound enzyme conjugates are measured optically. This procedure yielded the relation between signal intensity and HbA1c concentration shown in Fig. 7. At a total hemoglobin concentration of $30 \mu\text{g ml}^{-1}$ (456 nM), a reasonably linear dependence of absorbance on concentration is obtained in the range of 5–50% HbA1c. Furthermore, the authors reported no decrease in binding affinity of the glycated pentapeptide modified substrate to the anti-HbA1c antibodies even after being subjected to more than 20 repeated regeneration cycles. The authors did not present a similar diagram for their biosensor based on a cellulose membrane.

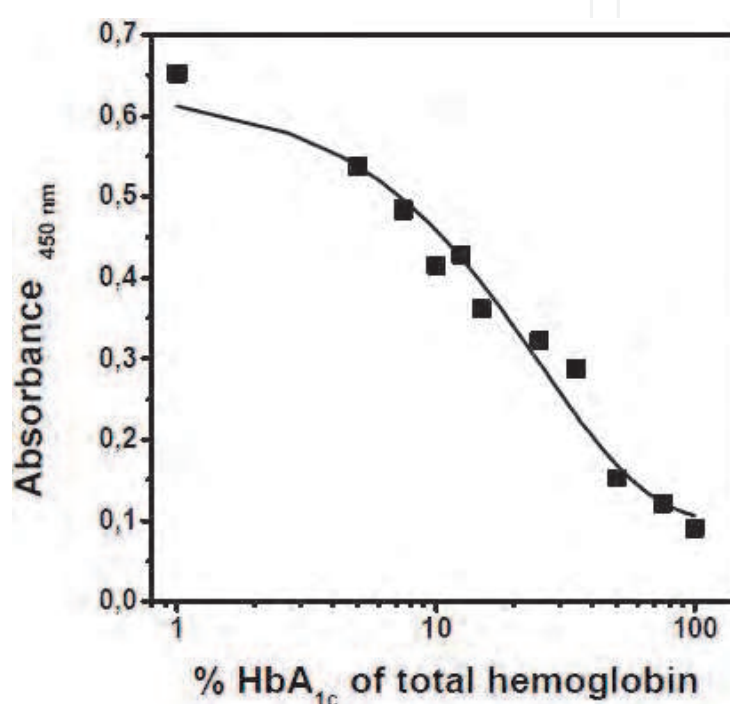


Fig. 7. Calibration curve for HbA1c measured with the Hemoglobin-A1c-ELISA at a final concentration of total hemoglobin of 30 mg ml^{-1} (465 nM) (Stöllner, Warsinke, Stöcklein, Dölling, & Scheller, 2001).

In a subsequent study, Stöllner et al and co-workers modified this biosensor for use as an amperometric immunosensor (Stöllner, Stöcklein, Scheller, & Warsinke, 2002). Their system works in 2 steps: selective enrichment of total hemoglobin on the surface of an affinity matrix followed by specific detection of immobilized HbA1c using a GOx-conjugated anti-HbA1c antibody. The affinity matrix consists of a cellulose membrane (fixed to a platinum surface) covalently immobilized by either haptoglobin (strong hemoglobin-binding protein) or anti-hemoglobin antibody. In this way, the surface of the biosensor becomes saturated with a variety of hemoglobin and HbA1c-type compounds that can be detected by amperometric (or optical) measurement of enzymatically produced H_2O_2 from GOx labels (Fig. 8). Electrochemical measurement was done in a PBS electrolyte after allowing the system to reach equilibrium after a potential of +600 mV vs. Ag/AgCl was applied. By preparing HbA1c samples with known concentrations in 3% BSA/PBS (blocking buffer), they did not have to determine the total hemoglobin concentration.

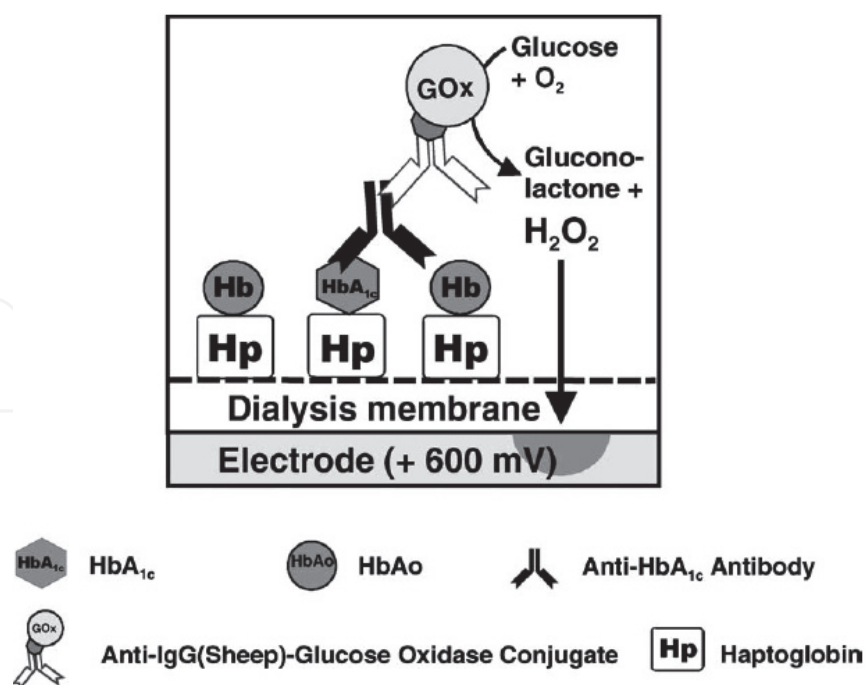


Fig. 8. Principle of the electrochemical HbA1c immunosensor (Stöllner, Stöcklein, Scheller, & Warsinke, 2002).

Their ELISA analysis showed a better reproducibility, higher sensitivity and signal-to-background ratio for the haptoglobin-based sensor than the one based on the anti-hemoglobin antibody. These researchers mentioned that this may be due to the more accessible glycated N-terminus of the β -chain of hemoglobin as a result of hemoglobin unfolding prior to the formation of a complex with haptoglobin. On the other hand, in the case of anti-hemoglobin antibody, the glycated N-terminal of the β -chain might be sterically hindered and less accessible for the anti-HbA1c antibody due to random orientation of hemoglobin molecules and a slight denaturation. As shown in Figs. 9 and 10, both ELISA and electrochemical analysis of HbA1c showed a linear correlation between %HbA1c of total hemoglobin and the signal (either absorbance or current) in the clinically relevant range of 5-20% HbA1c. The signal at 0% HbA1c corresponds to background effects. As can be seen, this background signal is relatively low in the case of the ELISA method, but comparable to the measurement obtained at 5% HbA1c using the electrochemical method. This background effect may be due to non-specific binding of the anti-HbA1c-GOx conjugate. The other problem with the electrochemical method is a standard deviation of 5-15% due to the use of one haptoglobin-modified membrane per sample in comparison to parallel screening with the ELISA method. Also, separate immunochemical reaction and indication steps of the bound GOx are required because of unspecific binding of the involved proteins to the plastic wall of the electrochemical cell. The time needed for HbA1c measurement in this work is approximately 3h due to non-optimized incubation times.

More recently, the same group published another study on an HbA1c biosensor based on electrochemical detection of ferroceneboronic acid (FcBA)-bound HbA1c (Liu, Wollenberger, Katterle, & Scheller, 2006). They introduced more electrochemical techniques in this approach. A zirconium dioxide nanoparticle-modified pyrolytic graphite electrode (PGE) was used in the presence of didodecyldimethylammonium bromide (DDAB) for total hemoglobin immobilization rather than a haptoglobin-modified cellulose membrane on a

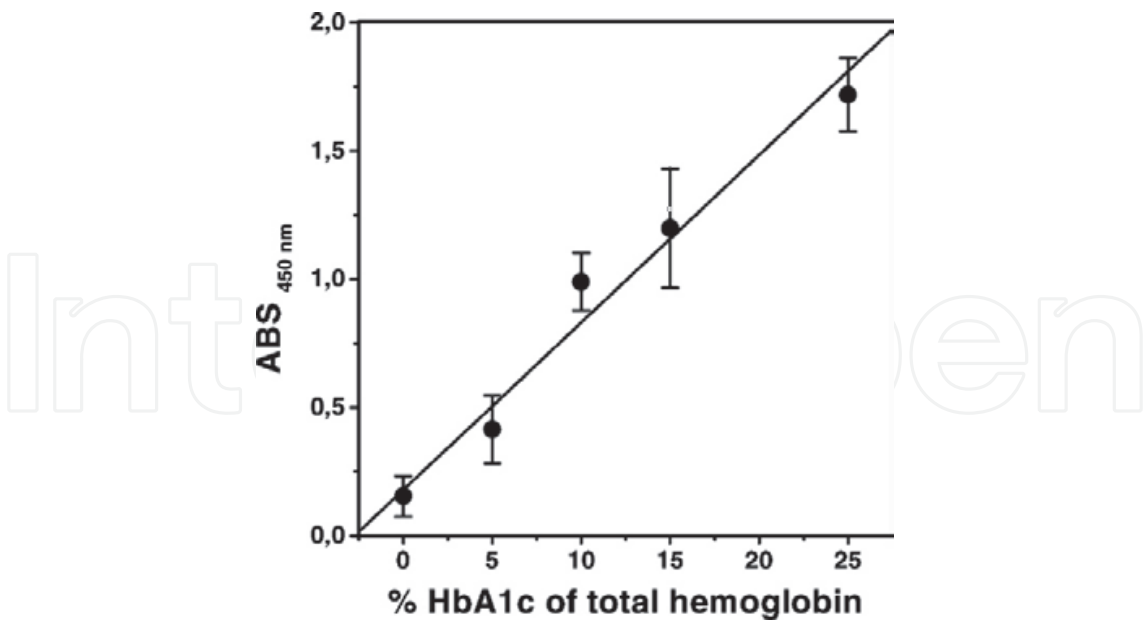


Fig. 9. Calibration curve for HbA1c measured with the sandwich immunoassay carried out on haptoglobin-modified cellulose membranes. The enzyme label GOx was detected optically (Stöllner, Stöcklein, Scheller, & Warsinke, 2002).

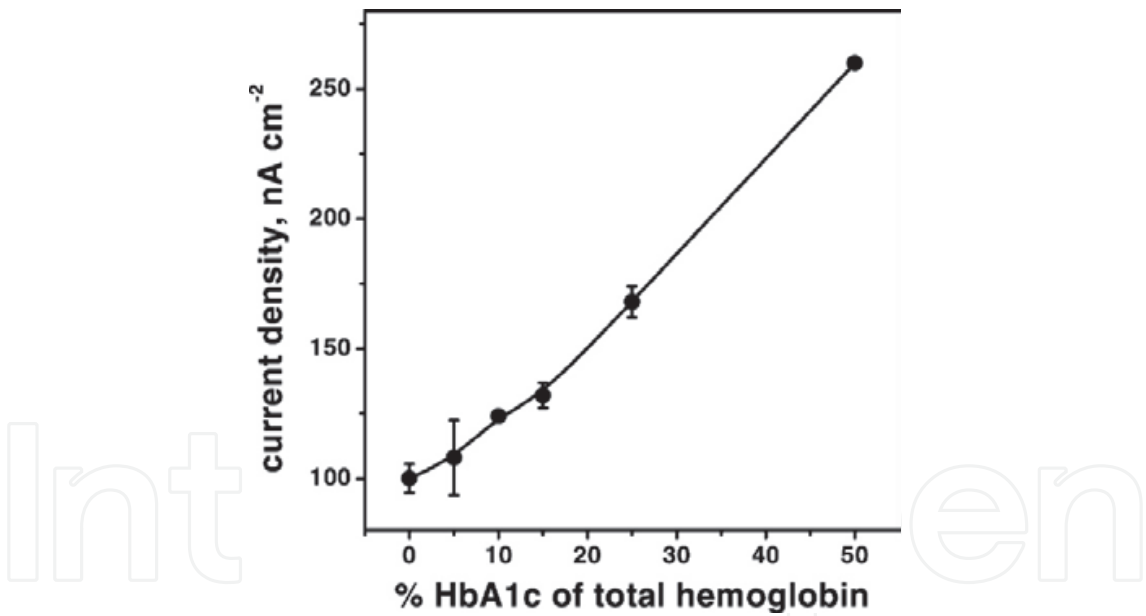


Fig. 10. Calibration curve for HbA1c using amperometric indication of the produced H₂O₂. The haptoglobin-modified cellulose membranes were fixed onto a Clark-type electrode (Stöllner, Stöcklein, Scheller, & Warsinke, 2002).

platinum electrode. Also, electrochemical measurement of HbA1c involved the use of FcBA instead of an anti-HbA1c-GOx conjugate. The PGE is used for protein (total hemoglobin) immobilization and DDAB accelerates electron transfer between hemoglobin and the electrode. Purified hemolysed erythrocytes from real human blood sample were mixed with the suspension of ZrO₂ nanoparticles in the DDAB solution and then applied to the electrode surface for total hemoglobin immobilization. Afterward, the electrode with

immobilized hemoglobin was incubated in FcBA solution for 30 min. The aromatic derivatives of boronic acid can react with 1,2- or 1,3-cis-diols to form reversible cyclic boronic esters in aqueous solutions under mild and easily controllable reaction conditions (Fig. 11). Consequently, FcBA serves 2 functions: selective binding to HbA1c over the other immobilized hemoglobins (using boronic acid part) and participation in the electrochemical reaction for HbA1c measurement through its ferrocene part. The total immobilized hemoglobin content was determined using cyclic voltammetry (CV) in pH 8.0 PBS solution, while the bound FcBA was detected using square wave voltammetry (SWV). SWV was used instead of CV since the chemically modified sensor with bound hemoglobin exhibited a relatively large charging current and higher sensitivity for the Fc label. The cyclic voltammogram obtained in the presence of hemoglobin-immobilized PEG showed 2 peaks related to the Fe(II)-Fe(III)-couple of the heme groups in hemoglobin. The hemoglobin concentration was obtained by integration of the reduction peak. Fig. 12 shows square wave voltammograms for a hemoglobin sensor obtained in solutions containing 2 different HbA1c concentrations before and after incubation in the presence of FcBA. The peak current increases significantly after incubation in the presence of FcBA and with increasing HbA1c percentage in total hemoglobin. Calibration curves for determination of %HbA1c at various total hemoglobin concentrations are presented in Fig. 13. From the point of view of sensitivity, the optimal total hemoglobin concentration is between 20-50 μ M. Measurement reproducibility of the fabricated sensor reported for 10.2% HbA1c samples at the different total hemoglobin concentrations was found to be 12.7% on average. Deviation of the HbA1c% measurements from the values obtained using the HPLC-based standard reference method was found to be quite high and vary from -10.7% to 31% for the 20 samples analyzed. The requirement for the separate determination of the total hemoglobin content also makes this an inconvenient aspect of this method.

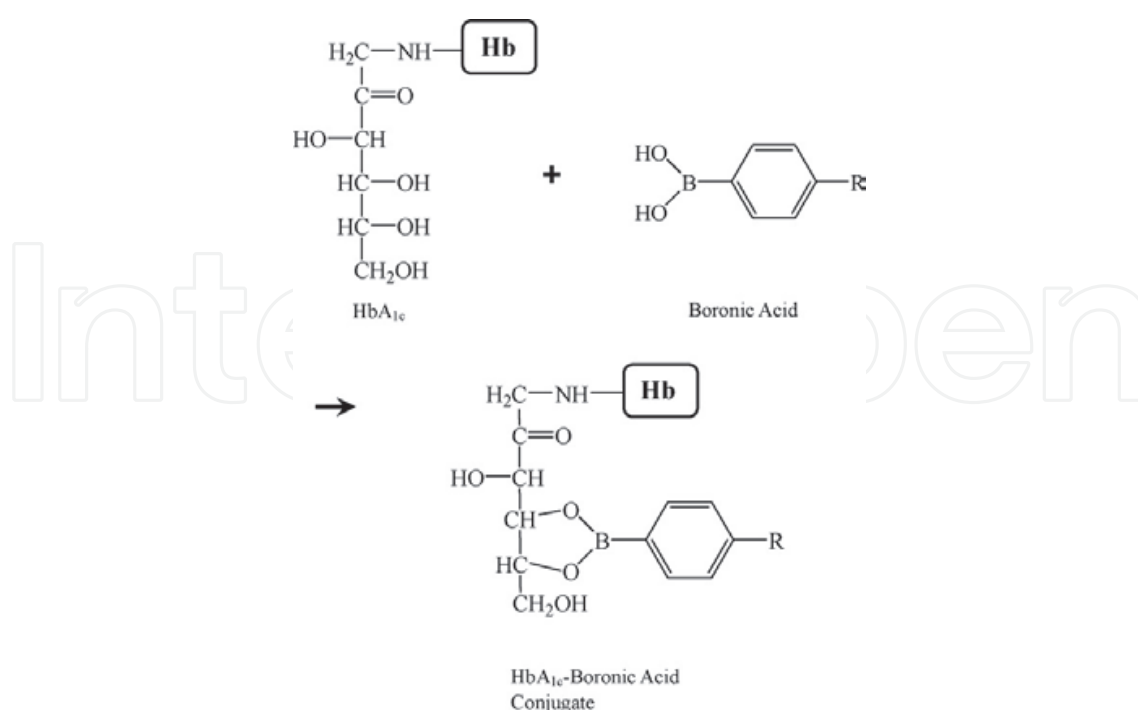


Fig. 11. Mode of conjugation between phenylboronic acid and protein HbA1c (Song & Yoon, 2009).

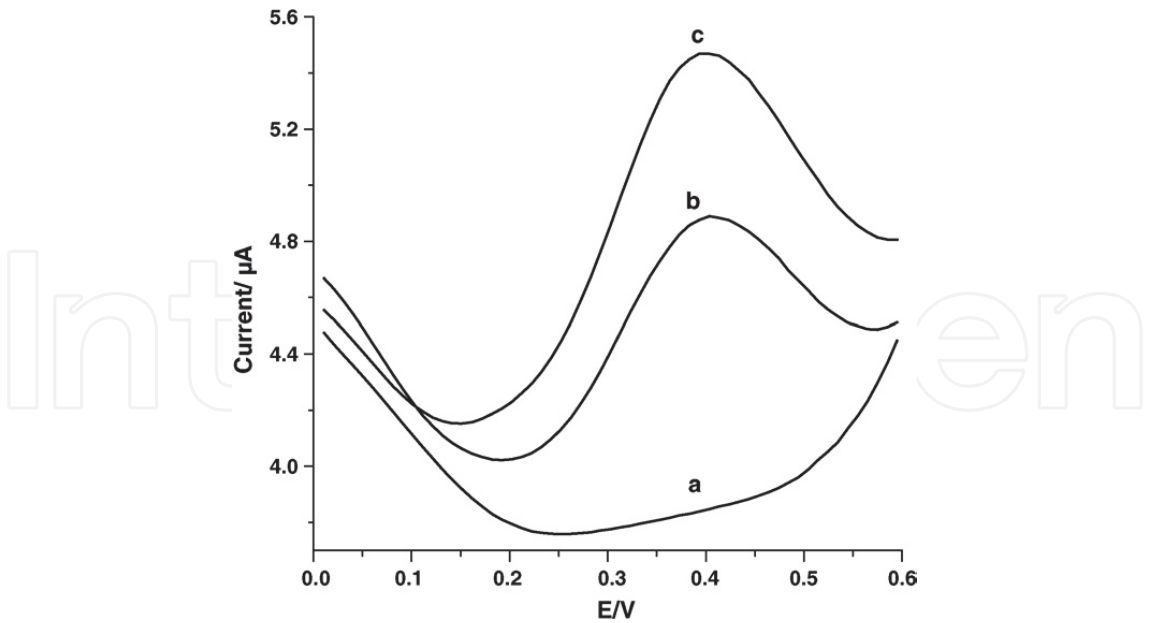


Fig. 12. Square wave voltammograms of a sensor containing 6.8% glycated hemoglobin before (a) and after incubation in FcBA (b) and Hb containing 14% glycated hemoglobin after incubation in FcBA (c) (Liu, Wollenberger, Katterle, & Scheller, 2006).

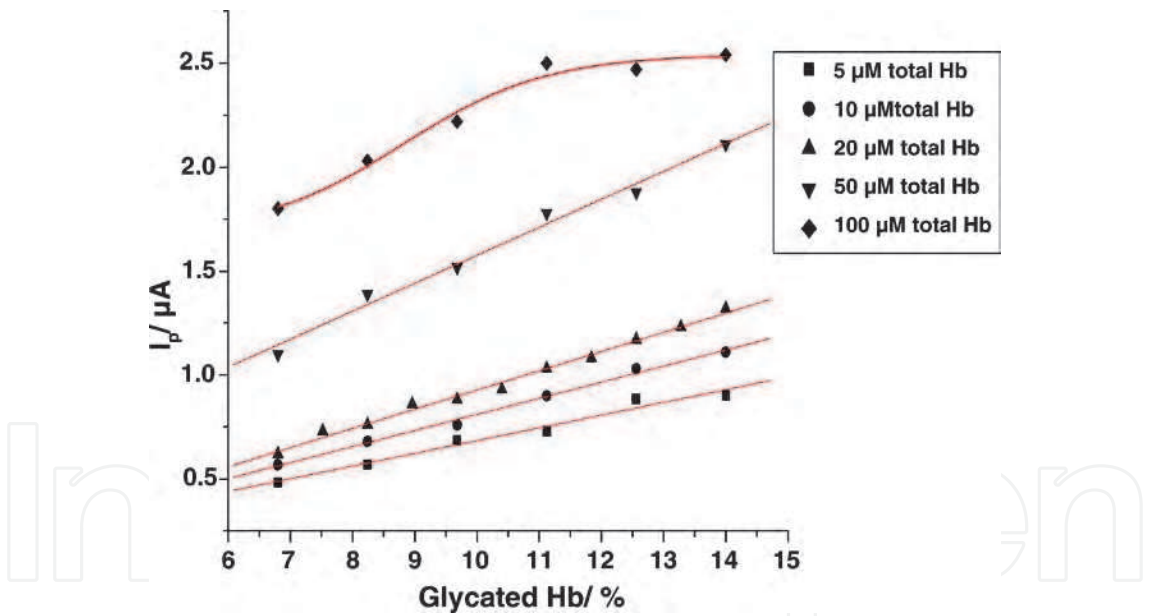


Fig. 13. Calibration curve for glycated hemoglobin determination for 3 μl 5 μM total Hb (■), 10 μM total Hb (●), 20 μM total Hb (▲), 50 μM total Hb (▼) and 100 μM total Hb (◆) (Liu, Wollenberger, Katterle, & Scheller, 2006).

More recently, Scheller et. al. further modified their previous amperometric HbA1c sensor into an electrochemical piezoelectric sensor (Halánek J. , Wollenberger, Stöcklein, & Scheller, 2007). The total hemoglobin content was determined using a mass-sensitive quartz crystal modified with a surfactant, while the FcBA-bound HbA1c on the surface was measured using square wave voltammetry. A piezoelectric quartz crystal was coated with gold and covalently modified with the surfactants. Of the four surfactants evaluated,

deoxycholate (DOCA) was found to be optimal with regard to hemoglobin surface loading, regeneration and direct reduction of the bound hemoglobin. Unlike their previous work, blood samples were first incubated with FcBA and then applied on the modified surface. The boronic acid/diol interaction is much faster in alkaline conditions; on the other hand, hemoglobin has lower stability at these pHs. Consequently, the optimum pH for incubation was found to be 8.0. Denaturation of hemoglobin before incubation with FcBA (by heat treating at 75 °C for 300s) is required for detection of HbA1c and the electrochemical response of the heme groups and also increases binding with DOCA-modified surface. The amount of the total hemoglobin bound to the surface is monitored by a quartz crystal nanobalance (QCN). Upon immobilization of hemoglobin on the electrode surface, the oscillation frequency of the quartz crystal decreases. The decrease in the frequency is proportional to the amount of adsorbed total hemoglobin. Fig. 14 shows a typical response of the QCN upon hemoglobin binding and regeneration of the DOCA-modified piezosensor. The oscillation frequency decreases after hemoglobin binding, but increases again after washing loosely bound hemoglobin and returns back to the baseline after regeneration and removal of bound hemoglobin. More than 30 binding-regeneration cycles were possible without loss of sensitivity.

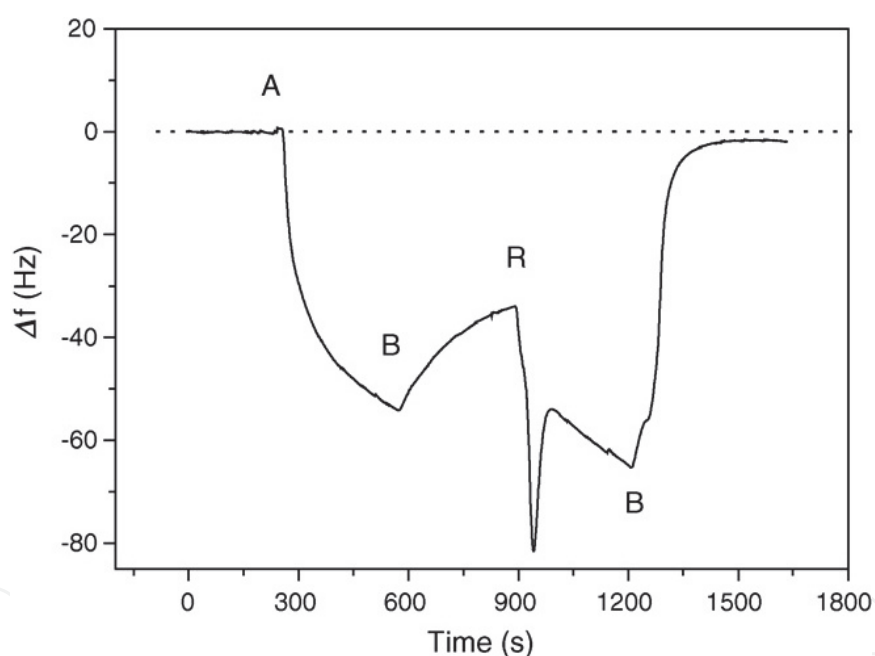


Fig. 14. Typical QCN response after Hb-binding to the DOCA-modified piezosensor. (A) Injection of Hb (7.75 μ M) is followed by (B) washing with buffer (Sørensen phosphate buffer pH 7.5) and (R) 5 min regeneration using pepsin solution. The dotted line represents the baseline of the piezoelectric quartz crystal. Before measurement, Hb was incubated at 75 °C for 300 s (Halánek J. , Wollenberger, Stöcklein, & Scheller, 2007).

These researchers used the same method of square wave voltammetry used in their earlier work for measurement of the FcBA-bound HbA1c (Fig. 15). To ensure that all HbA1c molecules are bound to FcBA, they added a 12-fold excess of FcBA to total hemoglobin. Fig. 16 shows the dependence of the current peak height of the SWV on %HbA1c. The standard deviation of this calibration curve obtained from 5 measurements of each sample is relatively high. This was partly attributed to the fact that the data were obtained in

experiments performed over a period of 5 days. Further optimization of the technique to reduce the measurement variability and attain a detection limit below 5% HbA1c is needed.

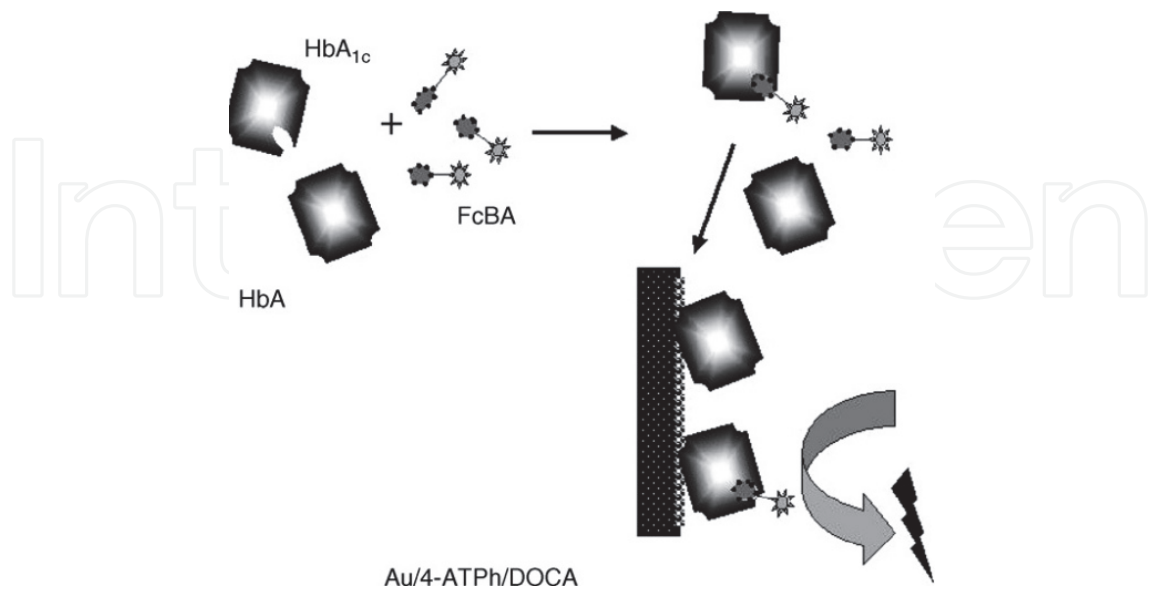


Fig. 15. Scheme of the electrochemical HbA1c sensor based on binding of FcBA-labelled HbA1c to the surface of the DOCA-modified piezoelectric quartz crystal and voltammetric read out of the label (Halámek J. , Wollenberger, Stöcklein, & Scheller, 2007).

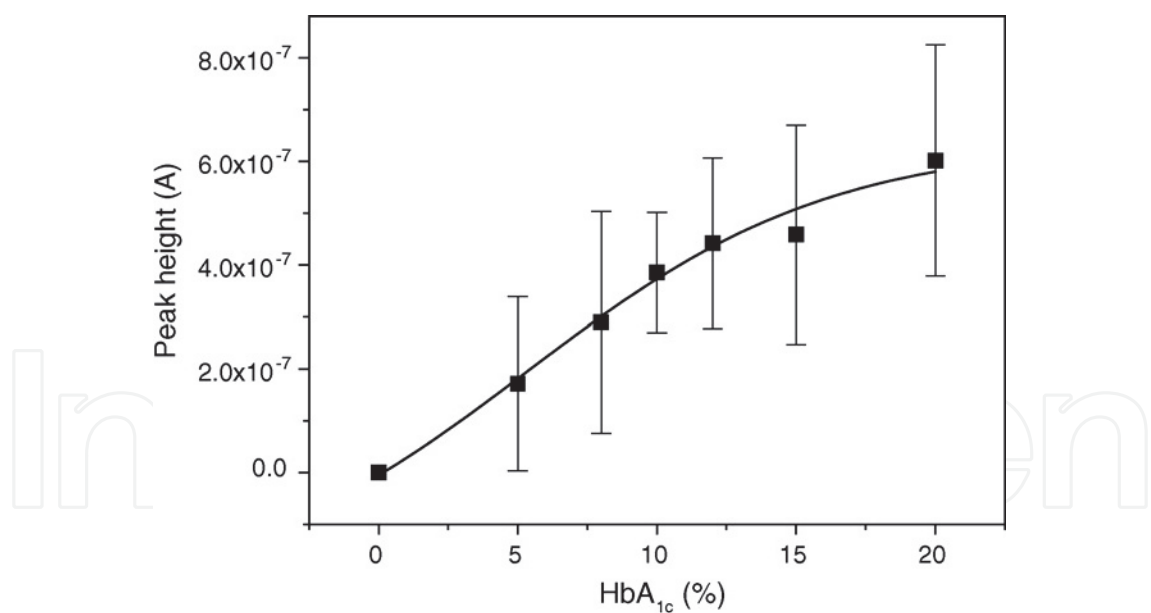


Fig. 16. Dependence of peak height of the SWV at +200mV vs. Ag/AgCl (1M KCl) on HbA1c content in Hb sample. Hb samples (7.75μM solution in Sörensen phosphate buffer pH 8.0) were preincubated with 1mMFcBA solution at 75 °C for 300 s (number of measurements per sample $n = 5$) (Halámek J. , Wollenberger, Stöcklein, & Scheller, 2007).

The same sensor was modified to enhance the signal by *in situ* tagging of an anti-HbA1c antibody with FcBA (Halámek J. , Wollenberger, Stöcklein, Warsinke, & Scheller, 2007). Measurement of the total immobilized hemoglobin was done by QCN as before, but an

additional step of incubating the anti-HbA1c antibody for 300s was done before introducing FcBA to the system. This antibody selectively binds to the glycosylated N-terminus of the β -chains of HbA1c. According to its structure, at least 5-6 terminal glycosylated residues contain vicinal cis-diol groups compared with 1-2 terminal sugar residues of the β -chains of HbA1c. Therefore, more FcBA per HbA1c molecule can bind to the surface and produce a higher SWV peak current and thereby increase the electrochemical signal. A comparison of this approach with that of direct tagging of HbA1c with FcBA described previously shows a 3.6-fold increase in sensitivity (Fig. 17). Although all the experiments were conducted in a single day, the standard deviations based on 3 measurements per sample were still high and accurate detection of HbA1c levels below 5% was still a problem.

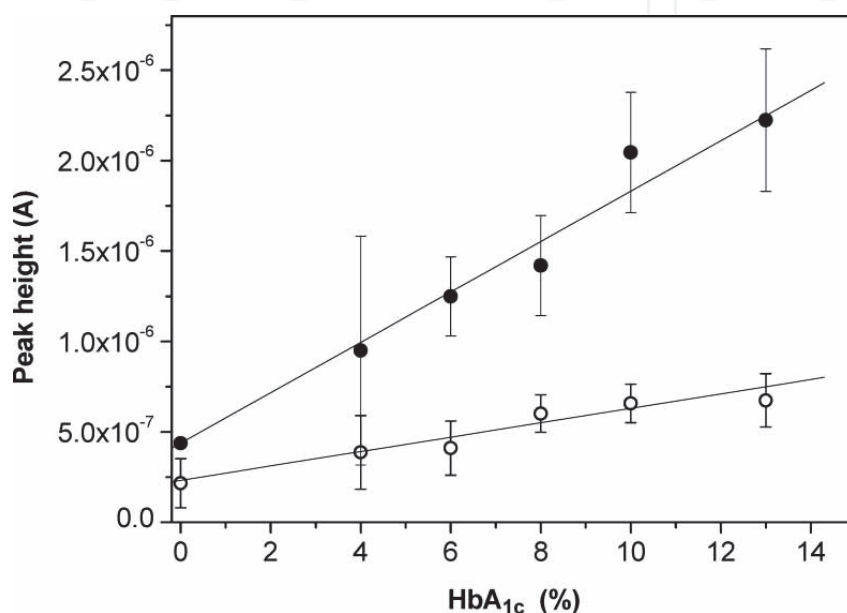


Fig. 17. Dependence of peak height of the SWV at +300 mV versus Ag/AgCl (1M KCl) on the HbA1c content in the Hb sample (total Hb 7.75 μ M in Sørensen buffer pH 8.0, preincubated at 75°C). After immobilization of Hb onto the DOCA sensor, either FcBA (○) or anti-HbA1c Ab and then FcBA (•) was injected. SWV were then measured in stopped flow (Halánek J., Wollenberger, Stöcklein, Warsinke, & Scheller, 2007).

Son et al fabricated a disposable biochip for electrochemical HbA1c measurement (Son, Seo, Choi, & Lee, 2006). They used ferricyanide ($K_3Fe(CN)_6$) as mediator so that the electrons released from the oxidation of Fe^{2+} in hemoglobin were transferred to the electrode by the ferricyanide/ferrocyanide couple. A schematic view of their %HbA1c measurement procedure is shown in Fig. 18. The components integrated in the system are a pair of interdigitated array (IDA) electrodes, HbA1c binding chamber, blood lysis chamber, filter, micro-pump and microchannel. After plasma separation (1) and red blood cell (RBC) lysis (2), the total hemoglobin stream branches off into two separate streams: in the lower stream HbA1c is immobilized on a packed agarose bead containing m-amino-phenylboronic acid (m-APBA) in the binding chamber and releases hemoglobin, while total hemoglobin flows in the upper stream (3). The ratio of the resulting electrochemical signals from the lower and upper streams after passing through the IDA electrodes yields the %HbA1c. Due to the non-homogeneous distribution of hemoglobin, the instantaneous current varies as a sample flows through the IDA electrodes. Consequently, the integral of the current over time was

used for measurement. Unfortunately, no information on the performance of this biosensor was provided in the article.

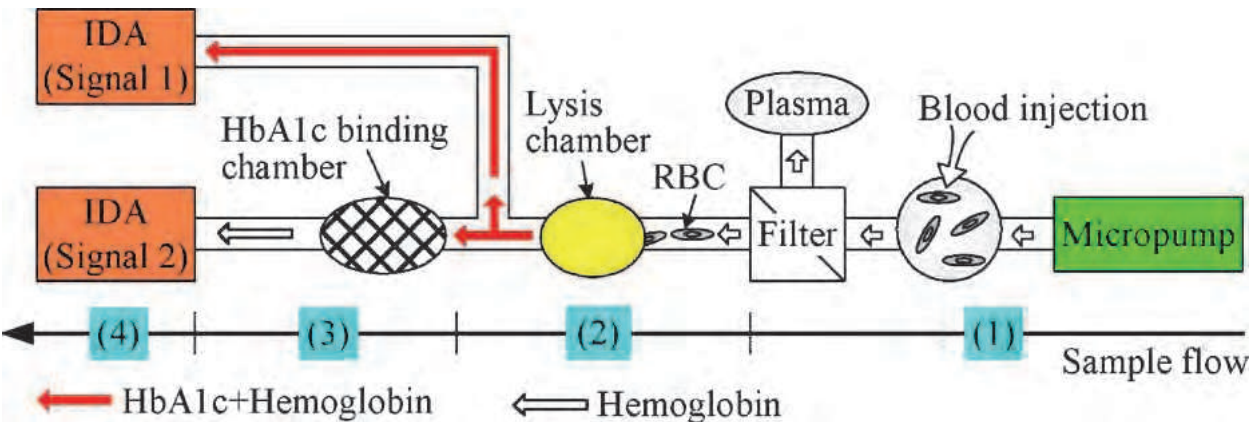


Fig. 18. Schematic of the %HbA1c measurement process (Son, Seo, Choi, & Lee, 2006).

In another study, Park et. al. reported an electrochemical HbA1c measurement method based on selective immobilization of HbA1c on a gold electrode covered with a thiophene-3-boronic acid (T3BA) self-assembled monolayer (SAM) and detecting HbA1c by label-free electrochemical impedance spectroscopy (EIS) (Park, Chang, Nam, & Park, 2008). Presumably, these researchers chose to modify the gold electrode with T3BA based on the common use of 3-aminophenylboronic acid to bind to a solid support for HbA1c separation from hemoglobin in boronate affinity chromatography. This species can form a self assembling monolayer (SAM) on a gold surface. The reported binding mechanism is based on bonding between the sulphur atom of the π -stacked thiophene SAM and the gold. The binding of T3BA and formation of a SAM on the gold was confirmed by the use of a quartz crystal microbalance (QCM), atomic force microscopy (AFM) and EIS experiments. Figs. 19 and 20 show the progress of T3BA binding over time as measured by QCM and an AFM image of a HbA1c/T3BA-SAM, respectively.

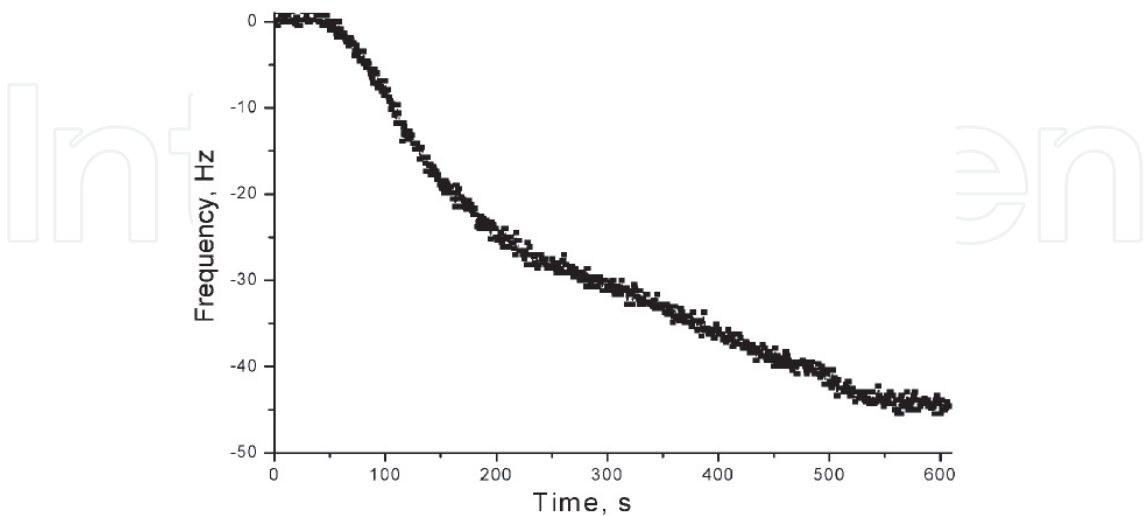


Fig. 19. QCM results for the HbA1c binding upon injection of 100 μ L of diluted 11.6% HbA1c solution into 2 mL of the pH 8.5 buffer solution (10 mM 4-ethylmorpholine) (Park, Chang, Nam, & Park, 2008).

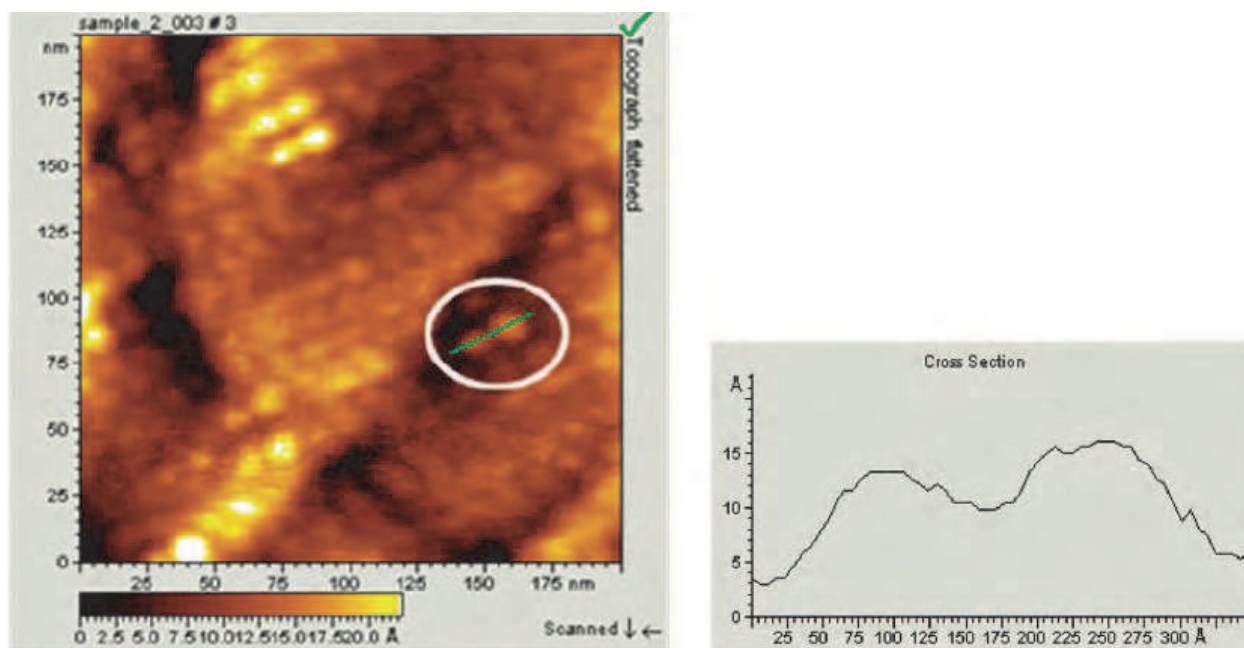


Fig. 20. AFM image the HbA1c/T3BA-SAM immobilized on it (left) along with corresponding cross-sectional profiles of the spots marked by white circles on the images (right) (Park, Chang, Nam, & Park, 2008).

Electrochemical determination of selectively immobilized HbA1c on the T3BA SAM is based on measuring the change in the capability of the gold electrode for electron transfer due to blocking of the electrode surface by HbA1c after immobilization. This is conducted using standard HbA1c solutions diluted with a buffered (pH 8.5) solution containing 10 mM 4-ethylmorpholine in a 3-electrode cell including a gold disk working electrode (0.020 cm²), Ag/AgCl reference electrode and platinum spiral wire counter electrode. The T3BA SAM has been found to have relatively high electrochemical activity since the charge transfer resistance R_{ct} is small only when it forms on the surface. On the basis of the shape of the EIS Nyquist plot obtained, the SAM appears to cover the electrode surface uniformly with no significant defects. The subsequent addition of HbA1c to the system causes the R_{ct} value to increase significantly. As shown in Fig. 21, the ratio of R_{ct} obtained in the presence of HbA1c to that obtained in its absence increases linearly with HbA1c concentration. Similarly, this ratio varies linearly with %HbA1c in samples with the same total hemoglobin concentration (Fig. 22). Such linear behaviour makes the T3BA-SAM modified electrode a satisfactory platform for a HbA1c sensor. On the other hand, these results indicate that the variation of this signal with HbA1c concentration also depends on total hemoglobin concentration. Consequently, the total hemoglobin concentration must also be determined to obtain the HbA1c content. Electrode regeneration can be carried out by washing with a sodium acetate buffer at pH 5.0. Since this method is not selective for HbA1c over glycated albumin (also present in blood under hyperglycemic conditions), glycated albumin must be separated from RBC by centrifugation.

In another study, Song and Yoon used a boronic acid-modified thin film interface for selective binding of HbA1c followed by electrochemical biosensing using an enzymatic backfilling assay (Song & Yoon, 2009). They used a freshly evaporated gold working electrode for the bottom-up layer formation process (Fig. 23). This procedure began with the formation of an amine-reactive DTSP SAM on the gold which was then transferred to a

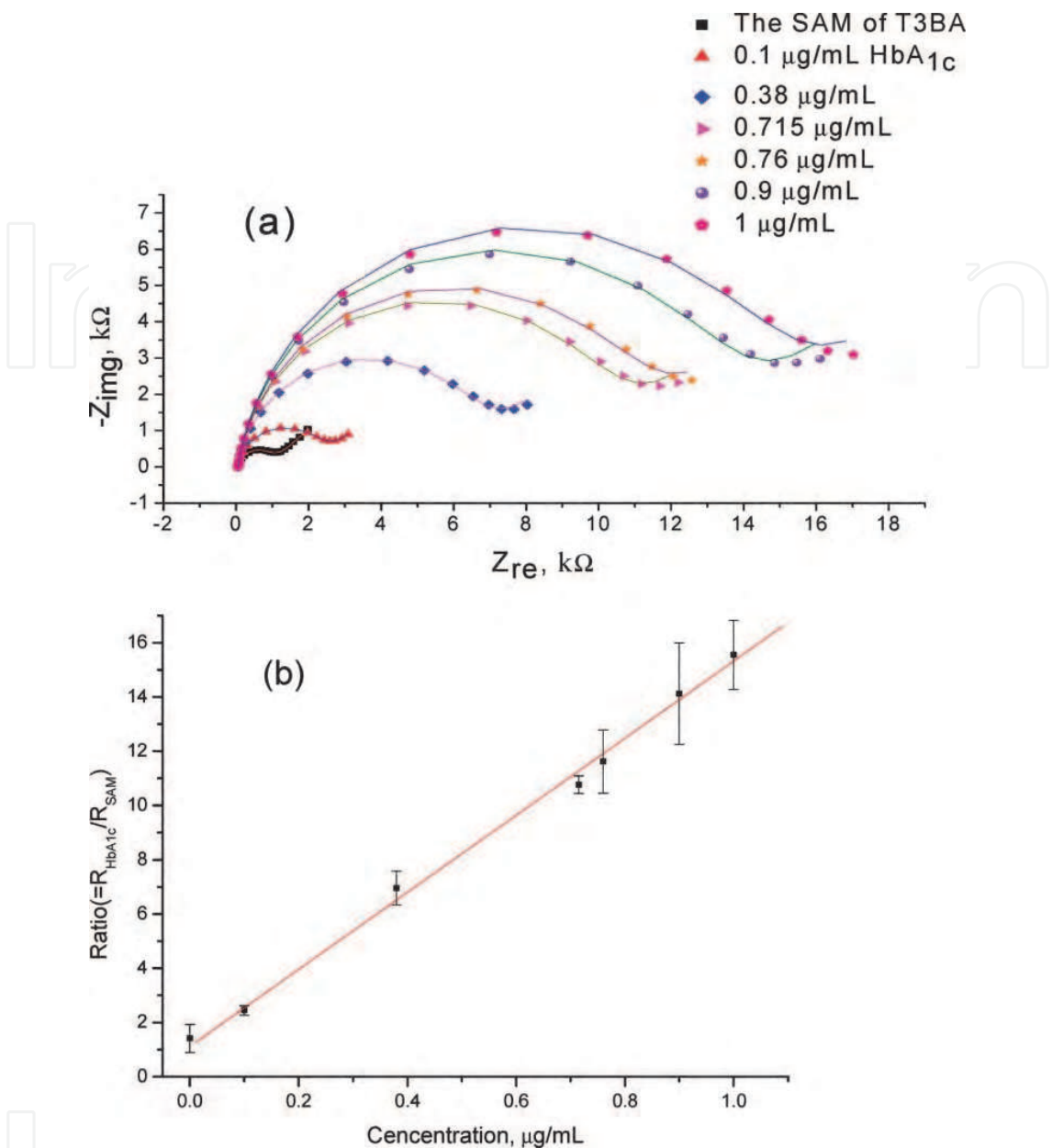


Fig. 21. (a) Impedance data obtained for the T3BA-SAM-covered electrode before and after immersion into various HbA1c concentrations diluted with 10 mM 4-ethylmorpholine buffer (pH 8.5) for 5 min. (b) The ratio of resistances plotted versus HbA1c concentration (μg/mL) (Park, Chang, Nam, & Park, 2008).

poly(amidoamine) G4 dendrimer solution. Then 4-formyl-phenylboronic acid (FPBA) was immobilized on the dendrimer layer selective for HbA1c. FPBA functionalization was confirmed by XPS and cyclic voltammetry. To carry out the backfilling assay, samples with various ratios of HbA1c/HbA0 (with normal adult human hemoglobin concentration i.e. 150 mg/ml) in a pH 9.0 bicarbonate buffer were contacted with the functionalized surface to react with FPBA for 1 hour. After rinsing with buffer and PBS, 1 mg/ml activated GOx in PBS was added in order to bind to the remaining unreacted amine groups on the dendrimer-FPBA layer or 30 minutes. The response of this electrode sensor was assessed by subjecting it to a voltammetric scan from 0 to +500 mV vs. Ag/AgCl at a rate of 5 mV/s in PBS in the

presence of 0.1 mM ferrocenemethanol (as mediator) and 10 mM glucose (as substrate). The anodic current measured at +400 mV was chosen as the sensor signal because of stable current at this potential in the voltammogram. Fig. 24(A) shows voltammograms obtained at different HbA1c concentrations. As expected, an increase in the HbA1c concentration leads to a decrease in the resulting current due to less available space for GOx on the electrode. The corresponding calibration curve for the anodic current at +400 mV as a function of HbA1c concentration is shown in Fig. 24(B). Although this sensor has the advantage of signal amplification without the need for pretreatment such as labelling or use of labelled secondary antibody, incubation of the hemoglobin sample and then GOx solution requires 1 hour and 30 minutes, respectively. In addition, the sensitivity at HbA1c levels below 5% is not sufficient.

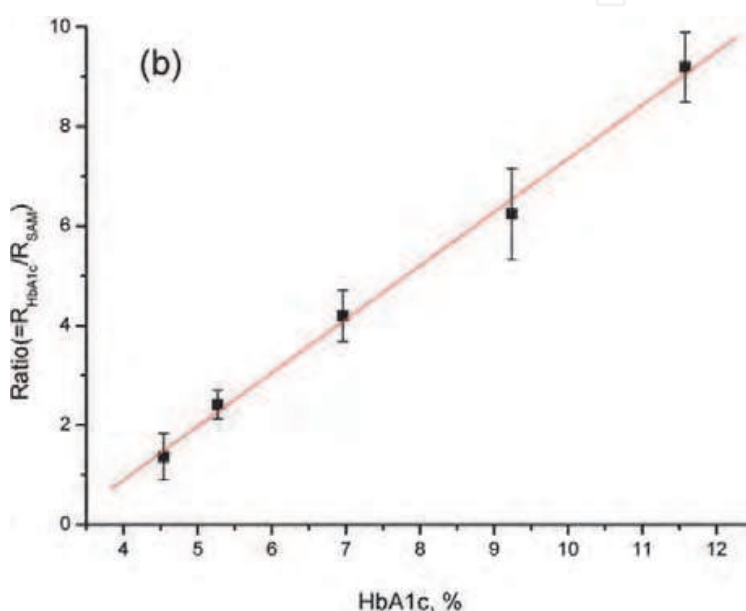


Fig. 22. R_{ct} ratio obtained at five HbA1c concentrations 20 minutes after sample injection (Park, Chang, Nam, & Park, 2008).

Qu and coworkers fabricated a micro-potentiometric Hb/HbA1c immunosensor based on an ion-sensitive field effect transistor (ISFET) using a MEMS fabrication process (Qu, Xia, Bian, Sun, & Han, 2009). Such ISFET biosensors have numerous advantages such as easy miniaturization and mass-production and rapid and label-free detection of a wide range of chemical and biochemical species. The procedure involved modification of the gold working electrode by electropolymerization of a polypyrrole (PPy)-HAuCl₄ composite followed by electrochemical synthesis of gold nanoparticles (AuNP) and modification of the gold reference electrode by applying a PPy film. The presence of AuNP on the surface (confirmed by FE-SEM) is reported to enhance antibody immobilization. Also, the PPy-AuNP electrode was electrochemically characterized by cyclic voltammetry and shown to exhibit better redox reaction reversibility than a PPy electrode. For hemoglobin and HbA1c immunosensor fabrication, anti-Hb antibodies and anti-HbA1c antibodies, respectively, were immobilized on the modified working electrodes. The fabricated microelectrode chip was then connected to an ISFET integrated chip. Charge adsorption at the ion/solid interface of the sensing layer leads to a potential drop and influences the gate voltage of the ISFET which is reflected by the change in the threshold voltage of the ISFET. Measurement of the hemoglobin level was done by successive injection of 10 μ L of hemoglobin solutions

with concentrations of 60-180 $\mu\text{g/ml}$ in PBS (pH 7.4) onto the SU-8 reaction pool of the sensor. Fig. 25 shows the change in differential voltage response (ΔE) upon successive addition of the samples (in comparison with the initial response in PBS). A linear relation between the hemoglobin concentration and voltage response is observed between 60 and 180 $\mu\text{g/ml}$. The corresponding sensor sensitivity and variation coefficient of ΔE was reported to be 0.205 $\text{mV } \mu\text{g}^{-1} \text{ ml}$ and 21%. A similar experiment on whole blood samples yielded a linear relation between ΔE and hemoglobin concentrations between 125-197 $\mu\text{g/ml}$ with a sensitivity of 0.20 $\text{mV } \mu\text{g}^{-1} \text{ ml}$.

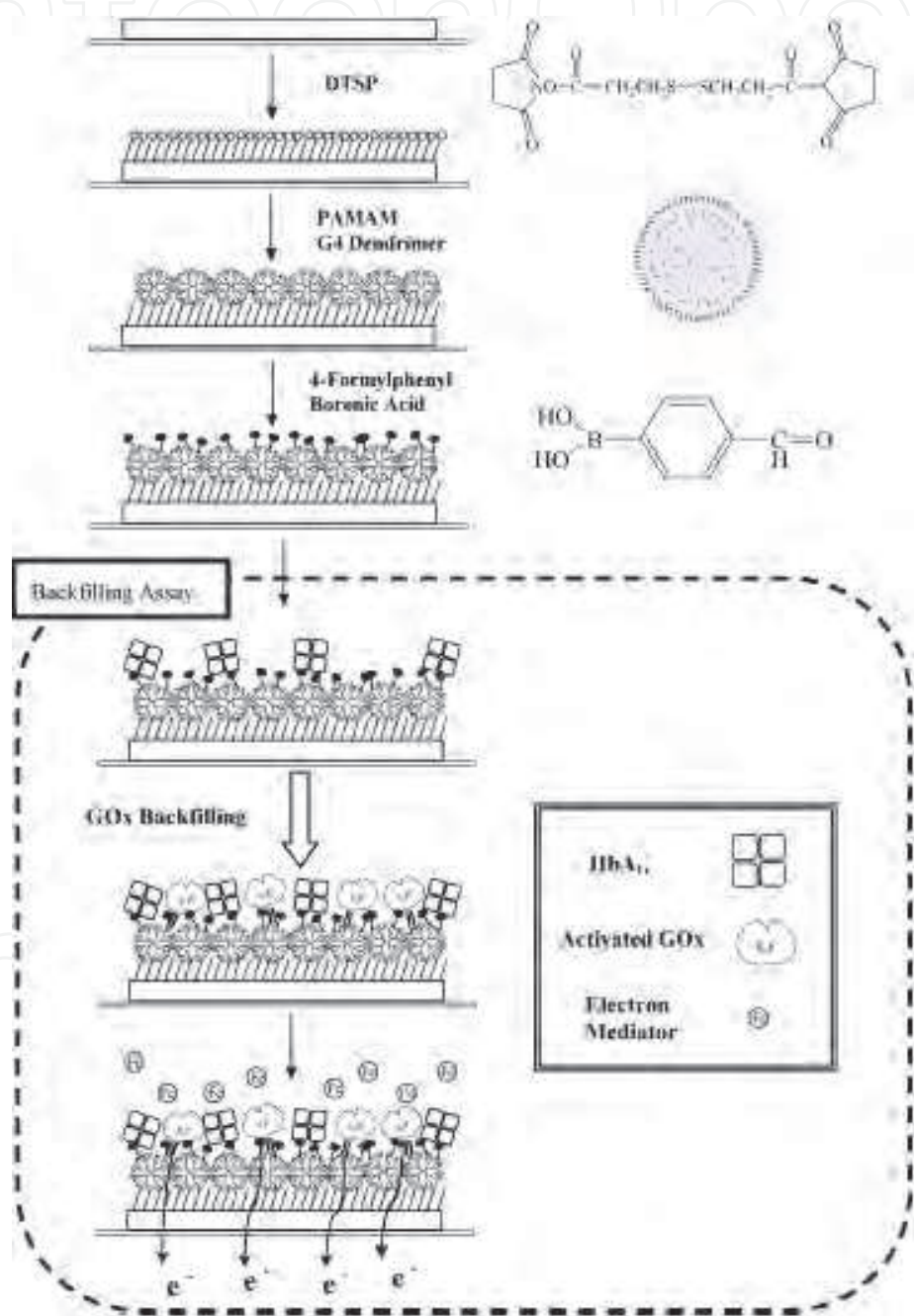


Fig. 23. Schematic diagram of “backfilling assay” between HbA1c and activated GOx. HbA1c binds to boronic acid and activated GOx binds to the remaining amine on the dendrimer monolayer (Song & Yoon, 2009).

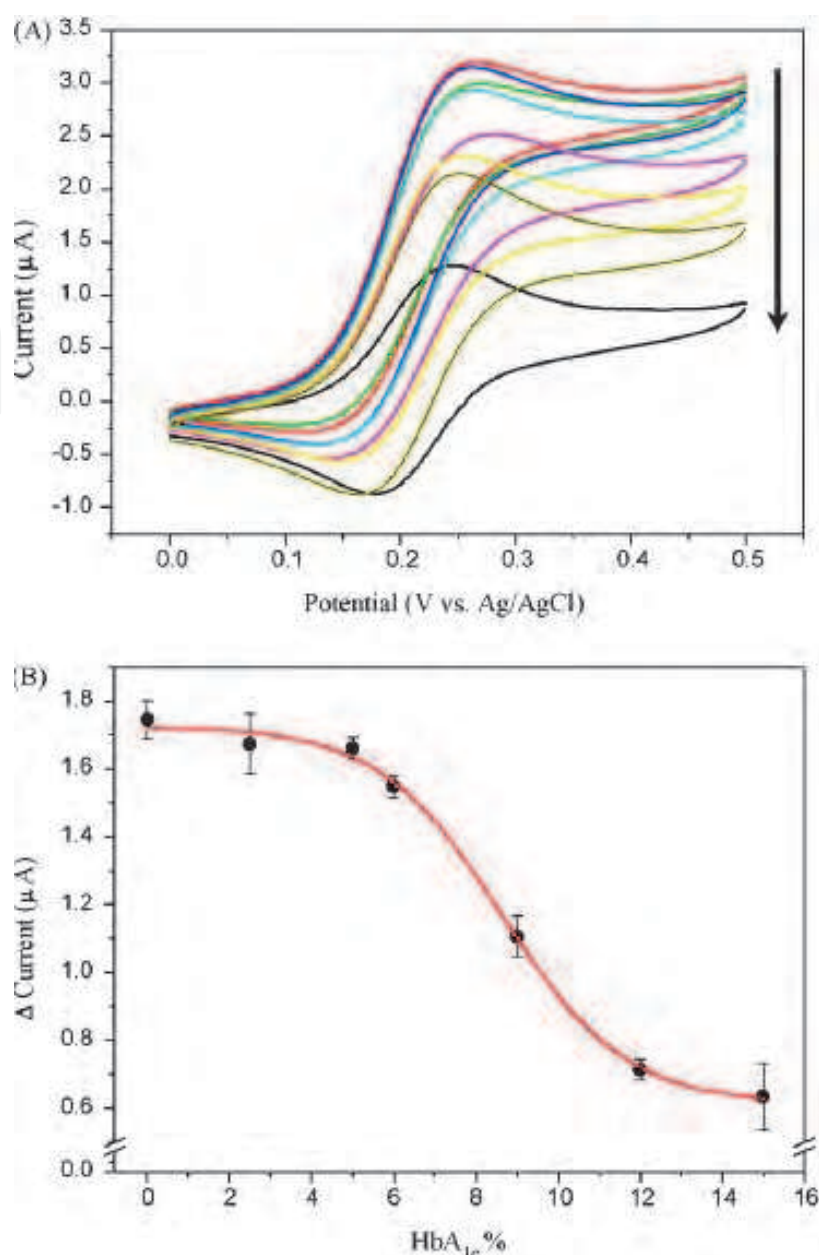


Fig. 24. Electrochemical biosensing of HbA1c by using Dend-FPBA electrodes. (A) Cyclic voltammograms of the backfilling assay between HbA1c and activated GOx at different HbA1c concentrations in the presence of ferrocenemethanol (0.1mM) in electrolyte with glucose (10mM) in 0.1MPBS (pH 7.2) at a 5mV/s sweep rate. A voltammogram before glucose addition is also included for comparison. (B) Calibration curve from the resulting backfilling assay as a function of target HbA1c concentration. Signal current levels were measured at +400mV from the background-subtracted voltammograms for respective analyte concentrations. The mean value from three independent analyses is shown at each concentration with error bar indicating the standard deviation (Song & Yoon, 2009).

The HbA1c concentration was measured using the same procedure on 10 μL solutions containing concentrations of 4-18 μg/ml HbA1c in PBS (pH 7.4) Fig. 26 shows a linear dose-response over this concentration range. Sensor sensitivity and variation coefficient of ΔE was reported to be 1.5087 mV μg⁻¹ ml and 24%. The change in response due to the addition

of potential interferents such as immunoglobulin G (100 $\mu\text{g/ml}$), α -fetoprotein (2.5 $\mu\text{g/ml}$) and BSA (1%) was found to be less than 9.2%. It was also found that the ΔE of the hemoglobin sensor decreased about 33.2% after storage at 4°C under dry conditions for 5 days in 100 $\mu\text{g/ml}$ hemoglobin in PBS (pH 7.4). The same trend was observed for a HbA1c sensor which showed a decrease in ΔE by about 35.1% after storage at 4°C under dry conditions for 5 days in 8 $\mu\text{g/ml}$ hemoglobin in PBS (pH 7.4). This change in response was attributed to the slow deactivation of antibodies during storage. Although this sensor has a short response time (less than 1 min) in comparison to other HbA1c biosensors and low fabrication costs (in the case of batch produced electrode chips), its low stability and the relatively high variability of its signal are problems requiring further improvement.

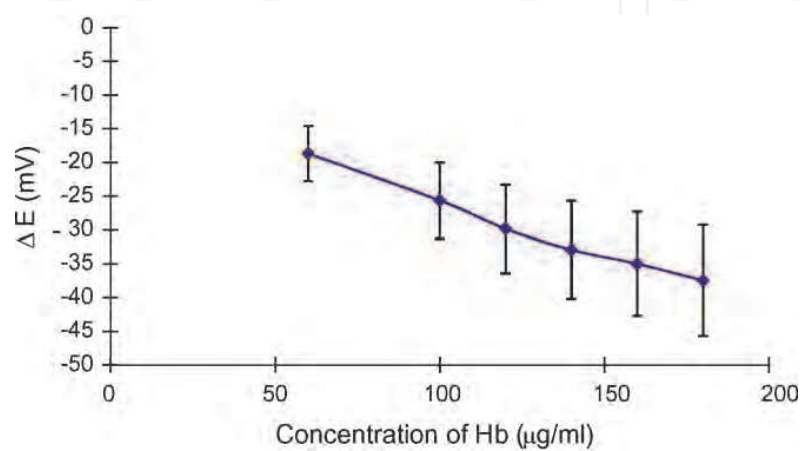


Fig. 25. Differential voltage response of the ISFET hemoglobin immunosensor to successive injections of Hb solutions with concentrations of 60, 100, 120, 140, 160 and 180 $\mu\text{g/ml}$ in PBS (pH 7.4). The coefficient of variation of the change of voltage response ΔE was 21% for measurements with three independently prepared electrodes. Voltages were measured 60 s after sample injection (Qu, Xia, Bian, Sun, & Han, 2009).

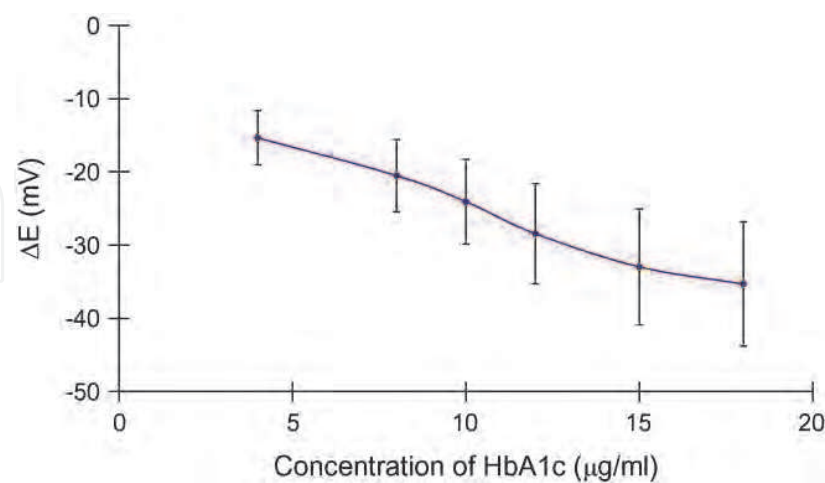


Fig. 26. Differential voltage response of the ISFET hemoglobin-A1c (HbA1c) immunosensor to successive injections of 4, 8, 10, 12 and 15 $\mu\text{g/ml}$ HbA1c solution in PBS (pH 7.4). The coefficient of variation for the change of voltage response ΔE was 24% for measurements with three independently prepared electrodes. Reported voltages were taken 60 s after HbA1c injection (Qu, Xia, Bian, Sun, & Han, 2009).

The same group further extended their approach by using SAMs (Xue, Bian, Tong, Sun, Zhang, & Xia, 2011). They designed a micro-potentiometric immunosensor based on mixed SAMs containing an array of gold nanospheres (instead of a PPy-AuNP layer) for HbA1c measurement (Fig. 27). The surfaces of nano-gold particles and a gold electrode were both modified by SAMs. This modification was done to address some of the problems associated with the use of nanoparticles in immunosensor fabrication. It also plays a role as an insulating film which is suitable for a FET, stabilizes covalent immobilization of antibodies and can eliminate the nonspecific sites to prevent noise interferences. The two-layer structure of SAMs with different chain lengths also helps reduce steric hindrance.

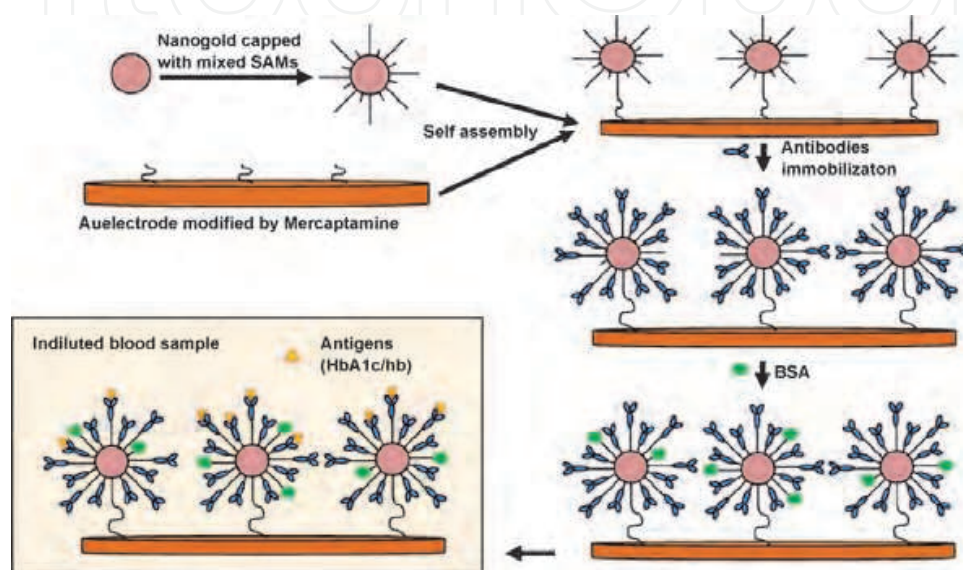


Fig. 27. Schematic diagram of electrode modification process and specific binding in diluted blood sample (Xue, Bian, Tong, Sun, Zhang, & Xia, 2011).

The electrode surface was modified by combining AuNPs with a mixed thiol solution (10 mM of both 16- and 3- mercaptohexadecanoic acid in ethanol) to form a two-layer SAM on AuNP followed by covalent immobilization on a gold electrode already modified with mercaptoethylamine-SAM using NHS and EDC. Antibodies were immobilized on the modified electrode using NHS and EDC as well. SEM images of the modified electrode showed a more uniform distribution of AuNPs which was attributed to the presence of SAMs. Electrochemical characterization of the modified electrode using CV and EIS confirmed that the SAMs had an insulating effect by decreasing the oxidation/reduction current and increasing the interfacial resistance. Also, the presence of AuNP increased the electrode sensitivity about 2-fold by raising the surface area-to-volume ratio of the sensor and making more sites available for antibody immobilization (Fig. 28A).

Measurements of hemoglobin and HbA1c content were conducted on 5 μL samples of simulated blood solution. Hemoglobin with concentrations of 166.67-570 ng/ml and HbA1c with concentrations of 1.67-170.5 ng/ml were analyzed. Figs. 28B and C indicate that linear relations between reagent dose and the electrode response were obtained over the concentration ranges from 166.67 to 570 ng/ml for hemoglobin and from 50 to 170.5 ng/ml for HbA1c. Sensor sensitivity was also reported to be 40.42 $\mu\text{V}/(\text{ngmL}^{-1})$ and 94.73 $\mu\text{V}/(\text{ngmL}^{-1})$ for hemoglobin and HbA1c, respectively. Also, the relative standard deviation of the measurements (RSD) was 5%. The good linearity of the results was attributed to the

absence of significant interferences from bovine serum albumin, lysis solution, potassium ions and chloride ions in the simulated blood sample as well as good biocompatibility of the method and a stable combination with antibodies. In comparison with their previous sensors based on mixed SAMs, the use of wrapped AuNP arrays increased the sensor sensitivity from the order of $\mu\text{g/mL}$ to ng/mL and lowered the standard deviation from above 20% to 5%, while reaching a dilution factor of 150,000 times.

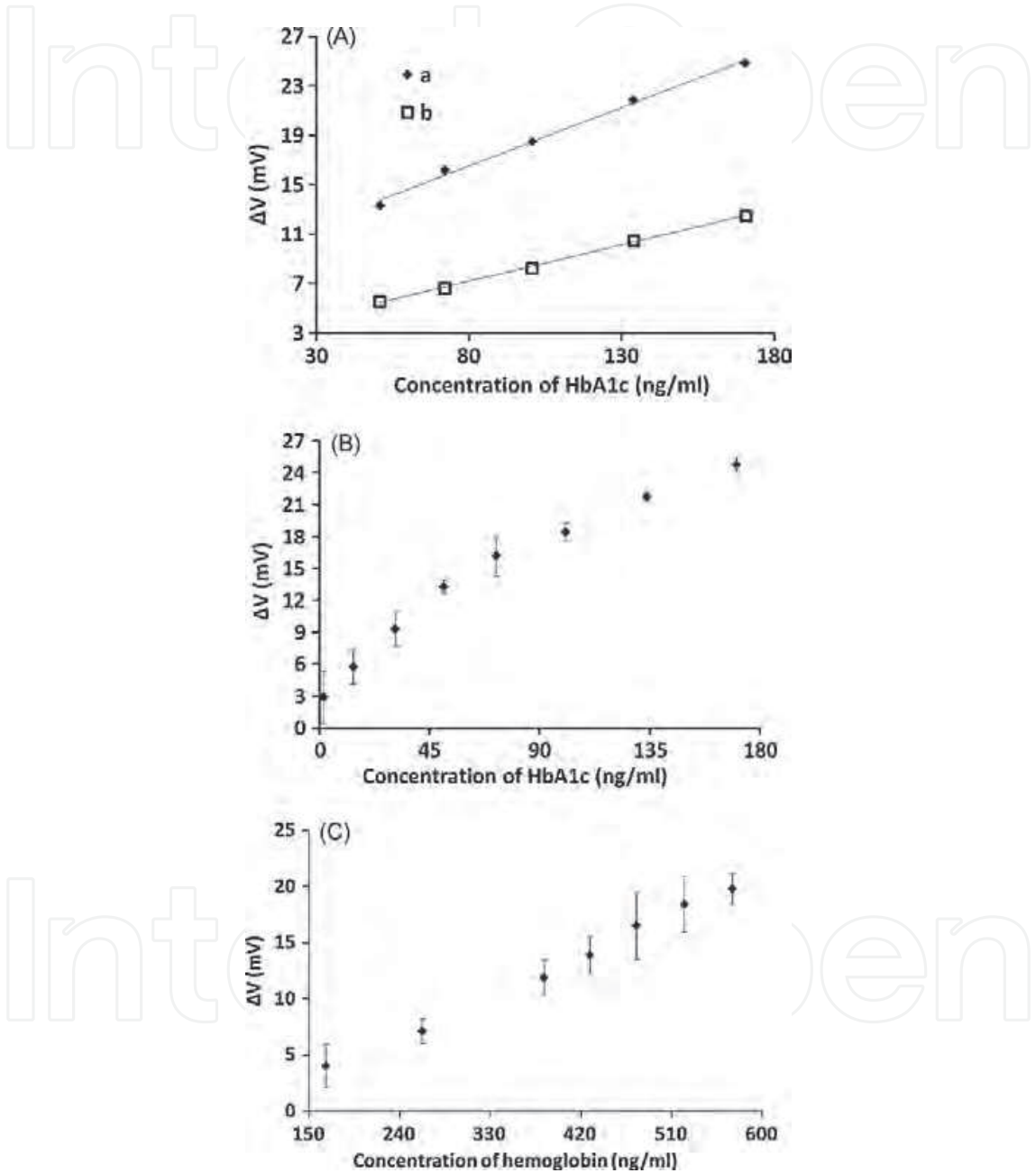


Fig. 28. Potential output of the immunosensor in a phosphate buffer solution of pH7.4 in the presence of simulated blood samples containing different concentrations of HbA1c and hemoglobin: (A) effect of HbA1c using two methods: (a) mixed SAM wrapped nano-spheres method and (b) mixed SAM method); (B) response to HbA1c; (C) response to hemoglobin. The results are the mean values of 3 measurements (Xue, Bian, Tong, Sun, Zhang, & Xia, 2011).

4. Conclusion

HbA1c point-of-care (POC) devices can potentially play an important role in diabetes diagnosis and management. However, they suffer from problems of low accuracy and reproducibility and so are not yet reliable enough to be recommended for clinical use at this time. This chapter reviews the research that has been done in the past decade or so to fabricate and improve the performance of HbA1c biosensors. A variety of approaches has been adopted to fabricate these sensors, making it difficult to compare them. However, based on the research to date, it appears that FV-based sensors require more steps for sample preparation, making their application in POC devices less favourable. Sensors that use label-free methods based on FET are less complicated for the user and require less time for measurement of HbA1c levels, but improvement to their sensitivity and especially reproducibility are needed in order to be accepted by clinicians and be suitable for introduction to the commercial market. Consequently, considerable work is still needed for the development of accurate, simple, reliable and cheap HbA1c biosensors.

5. Acknowledgment

Support for this research has been provided to two of the authors (PC and MP) by the Natural Sciences and Engineering Research Council of Canada (NSERC) and to one of the authors (PC) by the Canadian Foundation for Innovation (CFI) and the Canada Research Chairs (CRC) Program.

6. References

- Alexander, C., Andersson, H. S., Andersson, L. I., Ansell, R. J., Kirsch, N., Nicholls, I. A., et al. (2006). Molecular imprinting science and technology: a survey of the literature for the years up to and including 2003. *JOURNAL OF MOLECULAR RECOGNITION*, Vol. 19, pp. 106–180
- Berg, A. H., & Sacks, D. B. (2008). Haemoglobin A1c analysis in the management of patients with diabetes: from chaos to harmony. *Journal of Clinical Pathology*, Vol. 61, pp. 983–987
- Chien, H.-C., & Chou, T.-C. (2010). Glassy Carbon Paste Electrodes for the Determination of Fructosyl Valine. *Electroanalysis*, Vol. 22, No. 6, pp. 688 – 693
- Chuang, S.-W., Rick, J., & Chou, T.-C. (2009). Electrochemical characterisation of a conductive polymer molecularly imprinted with an Amadori compound. *Biosensors and Bioelectronics*, Vol. 24, pp. 3170–3173
- Fang, L., Li, W., Zhou, Y., & Liu, C.-C. (2009). A single-use, disposable iridium-modified electrochemical biosensor for fructosyl valine for the glycoslated hemoglobin detection. *Sensors and Actuators B*, Vol. 137, Vol. 235–238
- Halámek, J., Wollenberger, U., Stöcklein, W. F., Warsinke, A., & Scheller, F. W. (2007). Signal Amplification in Immunoassays Using Labeling via Boronic Acid Binding to the Sugar Moiety of Immunoglobulin G: Proof of Concept for Glycated Hemoglobin. *Analytical Letters*, Vol. 40, pp. 1434–1444
- Halámek, J., Wollenberger, U., Stöcklein, W., & Scheller, F. (2007). Development of a biosensor for glycated hemoglobin. *Electrochimica Acta*, Vol. 53, pp. 1127–1133

- Harris, M., & Zimmet, P. (1997). *Classification of diabetes mellitus and other categories of glucose intolerance* (Second Ausg.). (K. Alberti, P. Zimmet, & R. Defronzo, Hrsg.) Chichester: John Wiley and Sons Ltd.
- Kost, G. J. (2002). 1. Goals, guidelines and principles for point-of-care testing. In *Principles & practice of point-of-care testing* (S. 3–12). Lippincott Williams & Wilkins.
- Lenters-Westra, E., & Slingerland, R. J. (2010). Six of eight hemoglobin A1c point-of-care instruments do not meet the general accepted analytical performance criteria. *Clinical Chemistry*, Vol. 56, pp. 44–52
- Liu, S., Wollenberger, U., Katterle, M., & Scheller, F. W. (2006). Ferroceneboronic acid-based amperometric biosensor for glycated hemoglobin. *Sensors and Actuators B*, Vol. 113, pp. 623–629
- Nathan, D. M. (2009). International Expert Committee Report on the Role of the A1C Assay in the Diagnosis of Diabetes. *DIABETES CARE*, Vol. 32, No. 7, pp. 1327–1334
- NGSP. (June 2010). Abgerufen am 9. 1 2010 von <http://www.ngsp.org/CAC2010.asp>
- Park, J.-Y., Chang, B.-Y., Nam, H., & Park, S.-M. (2008). Selective Electrochemical Sensing of Glycated Hemoglobin (HbA1c) on Thiophene-3-Boronic Acid Self-Assembled Monolayer Covered Gold Electrodes. *Analytical Chemistry*, Vol. 80, pp. 8035–8044
- Pohanka, M., & Skládal, P. (2008). Electrochemical biosensors – principles and applications. *Journal of Applied Biomedicine*, Vol. 6, pp. 57–64
- Qu, L., Xia, S., Bian, C., Sun, J., & Han, J. (2009). A micro-potentiometric hemoglobin immunosensor based on electropolymerized polypyrrole-gold nanoparticles composite. *Biosensors and Bioelectronics*, vol. 24, pp. 3419–3424
- Sode, K., Ohta, S., Yanai, Y., & Yamazaki, T. (2003). Construction of a molecular imprinting catalyst using target analogue template and its application for an amperometric fructosylamine sensor. *Biosensors and Bioelectronics*, Vol. 18, pp. 1485–1490
- Sode, K., Takahashi, Y., Ohta, S., Tsugawa, W., & Yamazaki, T. (2001). A new concept for the construction of an artificial dehydrogenase for fructosylamine compounds and its application for an amperometric fructosylamine sensor. *Analytica Chimica Acta*, Vol. 435, 151–156
- Son, S. U., Seo, J.-H., Choi, Y. H., & Lee, S. S. (2006). Fabrication of a disposable biochip for measuring percent hemoglobin A1c (%HbA1c). *Sensors and Actuators A*, Vol. 130–131, pp. 267–272
- Song, S. Y., & Yoon, H. C. (2009). Boronic acid-modified thin film interface for specific binding of glycated hemoglobin (HbA1c) and electrochemical biosensing. *Sensors and Actuators B*, Vol. 140, pp. 233–239
- Stöllner, D., Stöcklein, W., Scheller, F., & Warsinke, A. (2002). Membrane-immobilized haptoglobin as affinity matrix for a hemoglobin-A1c immunosensor. *Analytica Chimica Acta*, Vol. 470, pp. 111–119
- Stöllner, D., Warsinke, A., Stöcklein, W., Dölling, R., & Scheller, F. (2001). Immunochemical Determination of Hemoglobin-A1c Utilizing a Glycated Peptide as Hemoglobin-A1c Analogon. *BIOSENSOR Symposium*. TÜBINGEN.
- Tsugawa, W., Ishimura, F., Ogawa, K., & Sode, K. (2000). Developement of an Enzyme Sensor Utilizing a Novel Fructosyl Amine Oxidase from a Marine Yeast. *Electrochemistry*, Vol 68, No. 11, 869–871

- Tsugawa, W., Ogawa, K., Ishimura, F., & Sode, K. (2001). Fructosyl Amine Sensing Based on Prussian Blue Modified Enzyme Electrode. *Electrochemistry* , Vol 69, No. 12, pp. 973-975
- Wang, J. (2006). *Analytical Electrochemistry*. United States of America: John Wiley & Sons, Inc.
- Wang, J. (2008). Electrochemical Glucose Biosensors. *Chemical Reviews* , Vol. 108, No. 2, pp. 814-825
- Xue, Q., Bian, C., Tong, J., Sun, J., Zhang, H., & Xia, S. (2011). A Micro Potentiometric Immunosensor for Hemoglobin-A1c Level Detection Based on Mixed SAMs Wrapped Nano-spheres Array. *Biosensors and Bioelectronics* , Vol. 26, pp. 2689-2693



Biosensors for Health, Environment and Biosecurity

Edited by Prof. Pier Andrea Serra

ISBN 978-953-307-443-6

Hard cover, 540 pages

Publisher InTech

Published online 19, July, 2011

Published in print edition July, 2011

A biosensor is a detecting device that combines a transducer with a biologically sensitive and selective component. Biosensors can measure compounds present in the environment, chemical processes, food and human body at low cost if compared with traditional analytical techniques. This book covers a wide range of aspects and issues related to biosensor technology, bringing together researchers from 16 different countries. The book consists of 24 chapters written by 76 authors and divided in three sections: Biosensors Technology and Materials, Biosensors for Health and Biosensors for Environment and Biosecurity.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Pu Chen, Mark Pritzker and Mohammadali Sheikholeslam (2011). Electrochemical Biosensor for Glycated Hemoglobin (HbA1c), Biosensors for Health, Environment and Biosecurity, Prof. Pier Andrea Serra (Ed.), ISBN: 978-953-307-443-6, InTech, Available from: <http://www.intechopen.com/books/biosensors-for-health-environment-and-biosecurity/electrochemical-biosensor-for-glycated-hemoglobin-hba1c->

INTech
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License](https://creativecommons.org/licenses/by-nc-sa/3.0/), which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.

IntechOpen

IntechOpen